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Los abajo firmantes, como Directores de esta Tesis, hacemos constar que la Tesis titulada "*Riesgos sanitarios asociados a la producción cinegética del jabalí: la enfermedad de Aujeszky*", y realizada por **José Francisco Ruiz Fons**, licenciado en Veterinaria, reúne los requisitos necesarios para su defensa y aprobación, y por tanto, para optar al grado de Doctor.

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El hombre es la medida de todas las cosas (Protágoras de Abdera)

En torno de la esencia está la morada de la ciencia (Platón)

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ÍNDICE GENERAL

OBJETIVOS

Objetivo 1. Determinar la situación sanitaria de las poblaciones peninsulares de jabalí con respecto al virus de la enfermedad de Aujeszky.

Objetivo 2. Establecer el riesgo que suponen los actuales sistemas de producción cinegética para las enfermedades, tomando como modelo el jabalí y la enfermedad de Aujeszky.

Objetivo 3. Determinar la presencia de otros agentes infecciosos en las poblaciones peninsulares de jabalíes y analizar su influencia sobre la función reproductora del jabalí.

Objetivo 4. Establecer el riesgo epidemiológico del jabalí como reservorio del virus de la enfermedad de Aujeszky para el porcino doméstico en el centro-sur de España.

Objetivo 5. Caracterizar epidemiológicamente los factores individuales, de manejo y del medio que influyen sobre la distribución del virus de la enfermedad de Aujeszky en el jabalí, así como establecer los patrones geográficos de distribución del virus.

Objetivo 6. Determinar la eficacia de métodos de control del virus de la enfermedad de Aujeszky en el jabalí, en concreto de la vacunación.

ORGANIZACIÓN DE LA TESIS

El trabajo de tesis se ha estructurado de forma lógica siguiendo un primer paso introductorio y de puesta en escena de la situación de las enfermedades víricas en las poblaciones de jabalí y el papel que puede jugar este suido silvestre como reservorio de estas enfermedades para el porcino doméstico; en segundo lugar se incluye el análisis descriptivo de la situación del virus de la enfermedad de Aujeszky en las poblaciones peninsulares de jabalí mediante estudios serológico y molecular; en un tercer paso se evaluó el efecto del virus de la enfermedad de Aujeszky y otros agentes infecciosos sobre la función reproductora de las hembras de jabalí; en cuarto lugar se analizó el riesgo epidemiológico del jabalí como reservorio para el cerdo doméstico en Castilla-La Mancha; en último lugar se realizó una vacunación experimental en jabalíes con la finalidad de determinar la plausibilidad de este método para el control del virus de la enfermedad de Aujeszky en el jabalí.

La estructura esquemática y el título de los apartados es el siguiente:

Capítulo 1. Introducción. Enfermedades víricas del jabalí: efectos en la dinámica poblacional y el papel como reservorio del jabalí.

Capítulo 2. Distribución y situación del virus de la enfermedad de Aujeszky en las poblaciones de jabalí de la España peninsular:

2.1. Estudio serológico del virus de la enfermedad de Aujeszky en el jabalí en España.

2.2. Patrones de infección del virus de la enfermedad de Aujeszky en el jabalí.

Capítulo 3. Seroprevalencia de seis patógenos reproductivos en jabalí (*Sus* scrofa) en España: el efecto sobre la función reproductora en las hembras de jabalí.

Capítulo 4. Interacciones epidemiológicas sobre la enfermedad de Aujeszky entre el porcino doméstico y el jabalí en Castilla-La Mancha, España.

Capítulo 5. Respuesta de anticuerpos en rayones (*Sus scrofa*) vacunados contra el virus de la enfermedad de Aujeszky.

Capítulo 6. Síntesis y conclusiones.

ABORDAJE DE LOS OBJETIVOS

Objetivo 1. Este objetivo ha sido cumplido en el **Capítulo 2**. Tanto las prevalencias de anticuerpos como la tasa de infección por el virus de la enfermedad de Aujeszky son muy elevadas en las poblaciones del centro y sur de la Península Ibérica. El virus no circula actualmente en las poblaciones de jabalí de la cornisa cantábrica.

Objetivo 2. Este objetivo está reflejado en los **Capítulos 2 y 3**. Los actuales sistemas de gestión cinegética tienen repercusión en los niveles de circulación del virus de la enfermedad de Aujeszky, así como en otros agentes infecciosos presentes en las poblaciones de jabalí. La probabilidad de un jabalí de ser infectado por estos agentes infecciosos es mucho mayor en las fincas manejadas que en aquellas que no se manejan. El notable incremento de las densidades de jabalíes en las fincas manejadas conlleva consigo un incremento en la tasa de contactos entre los animales, y por lo tanto, un incremento en los niveles de infección de los jabalíes sometidos a estos sistemas de manejo. Además, la tasa de circulación elevada de agentes infecciosos en los jabalíes de estas fincas puede reportar consecuencias sanitarias para los propios jabalíes, para otras especies silvestres, para los animales domésticos y para el ser humano. Así mismo, se observó que la tasa reproductiva de las hembras de jabalí mejoraba bajo situaciones de ausencia de manejo.

Objetivo 3. Este objetivo es abordado en el **Capítulo 3**. Otros agentes infecciosos están presentes en las poblaciones de jabalíes estudiadas en mayor o menor medida. Se determinó el posible efecto de la presencia de estos agentes infecciosos sobre parámetros reproductivos de las hembras de jabalí.

Objetivo 4. El objetivo ha sido cumplido y se refleja en el **Capítulo 4**. Se estudió la relación epidemiológica entre el jabalí y el cerdo doméstico en relación al virus de la

enfermedad de Aujeszky, concluyendo la ausencia de asociación entre ambos en el ámbito del área de estudio.

Objetivo 5. Este objetivo está reflejado en el **apartado 1 del Capítulo 2** y en el **Capítulo 4**. Se determinaron los factores epidemiológicos con efecto sobre la distribución y presencia del virus de la enfermedad de Aujeszky a nivel individual en los jabalíes del centro-sur de la península. Se observó un incremento de la tasa de circulación de anticuerpos contra el virus en las hembras y en los animales adultos. Así mismo, factores del manejo de los animales resultaron influyentes en las mayores tasas de circulación de anticuerpos. Se obtuvo información de la distribución espacial de las tasas de infección por el virus tanto en los jabalíes en fincas cinegéticas como en las explotaciones de porcino doméstico de la región de estudio.

Objetivo 6. Este objetivo se cumplió con los resultados obtenidos en el **Capítulo 5**. Se evaluó experimentalmente la eficacia de la vacunación de rayones con cepas atenuadas del virus de la enfermedad de Aujeszky en la respuesta inmune humoral específica. Así mismo, se estableció un protocolo de vacunación factible para los sistemas de producción de jabalí en condiciones controladas (granjas). Se concluyó que la vacunación podría ser un método adecuado para el control de la enfermedad en jabalíes bajo situaciones controladas.

Capítulo 1. Introducción

Enfermedades víricas del jabalí: efectos en la dinámica poblacional y el papel como reservorio del jabalí

Francisco Ruiz-Fons, Joaquim Segalés, Christian Gortázar

A review of viral diseases of the European wild boar: effects on population dynamics and the reservoir role of the wild boar

Enviado a: ***The Veterinary Journal***

Resumen

Las poblaciones de jabalí han experimentado un gran incremento en su número y en su distribución geográfica en las últimas décadas. Esto puede implicar un incremento en la circulación de agentes de enfermedad y el contacto potencial con los animales domésticos y el hombre. Las enfermedades pueden afectar la dinámica poblacional de la fauna silvestre, aunque los efectos de muchas enfermedades víricas sobre el jabalí son ampliamente desconocidos. Muchas de las enfermedades víricas presentes en las poblaciones de cerdo doméstico están también presentes en los jabalíes, y estos últimos pueden ser un reservorio de enfermedades. Este es el caso claro de la peste porcina clásica, pero el conocimiento sobre otras enfermedades víricas de relevancia es escaso, como las enfermedades circovirales porcinas o la hepatitis E. El presente trabajo revisa el conocimiento científico actual sobre los efectos de las enfermedades víricas sobre las poblaciones de jabalí, así como sobre el papel de los jabalíes como reservorio de enfermedades. Además, esta revisión enfatiza aquellas enfermedades víricas de importancia en el cerdo doméstico y en el jabalí incluidas como enfermedades de declaración obligatoria a la Oficina Internacional de Epizootías (OIE).

Abstract

Wild boar populations have experienced a worldwide increment in their numbers and geographical spread during the last decades. This in turn increases the circulation of disease agents and the potential contact with domestic animals and humans. Diseases can affect population dynamics of wildlife, but effects of most viral diseases on the European wild boar are largely unknown. Many viral diseases present in domestic pig populations are also present in wild boars, and these animals could be a disease reservoir. This is clearly the case for classical swine fever, but little is known about

many other relevant viral diseases such as porcine circovirus diseases or hepatitis E. This work reviews the current scientific knowledge on the effects of viral diseases on wild boar populations, and on the role of wild boars as disease reservoirs. Moreover, this review emphasizes on significant viral diseases of domestic swine and wild boar included as notifiable diseases by the Office International des Epizooties (OIE).

Keywords: Domestic pig; Population dynamics; Reservoir; Viral diseases; Wild boar.

Introduction

Different studies have evidenced that wild boar population density tend to increase worldwide (Saez-Royuela and Tellería, 1986; Gortázar et al., 2000; Acevedo et al., 2006a). Higher abundances not only mean a larger number of hosts available for any transmissible disease, they also mean a proportionally higher contact rate between hosts, and hence greater possibilities for disease transmission (Acevedo et al., 2006b). The knowledge on the diseases present in wildlife populations is of concern for wild boar health, conservation purposes, and livestock production, as well as public health. Therefore, we aimed to review the knowledge about viral diseases of the wild boar, emphasizing on the effect of viral diseases on wild boar populations and on the role that wild boars could play as viral disease reservoir for domestic pigs. Special attention is paid to diseases notifiable to the Office International des Epizooties (OIE). Wild boar distribution and ecological features that would be of importance for a better understanding of the effects of viral diseases in wild boar population dynamics and pathogen circulation among wild populations are also reviewed.

Wild boar distribution and population dynamics

Wild boars naturally inhabit vast areas of Europe and North Africa and extend to Sri Lanka, Indonesia, Japan, Taiwan and Korea (Lever, 1994). As a consequence of

introductions, the wild boar also inhabits areas far away from its original distribution such as the south of the USA, some African countries, several Caribbean islands, Central and South America, Oceania, and many Southeast Asia and Pacific islands (Lever, 1994). In most of the areas where the wild boar has been introduced, hybridization with free-roaming domestic pigs has lead to a crossbreed that is frequently named as feral pig, feral swine or feral hog; both the feral pig and the European wild boar are the same species, *Sus scrofa*. Feral pigs are common in the south of the USA, Australia and New Zealand (Mayer and Brisbin, 1991; Oliver and Brisbin, 1993; Waithman et al., 1999; Wooddal, 1983). The wild boar was accidentally reintroduced to England through escapes from captive individuals, and two populations have established in the south of Great Britain (Goulding et al., 2003).

Along the text, "wild boar" will be used either for European wild boar and feral pig; although in specific cases European wild boar and feral pig will be distinguished as a consequence of their areas of distribution.

Although wild boars disappeared from many parts of Europe by the end of the 17th century due to hunting pressure and habitat degradation (Harting, 1880; Tisdell, 1982), their numbers have increased during the second half of the 20th century (Saez-Royuela and Tellería, 1986; Gortázar et al., 2000; Acevedo et al., 2006a). This is mainly due to significant habitat changes, along with a reduction in the hunting pressure, together with a high reproductive rate and the increase of restocking practices for hunting purposes (including food supplementation). Moreover, wild boar populations have increased not only in Europe but also in other areas where they were introduced (Hahn, 1997; Romero et al., 1997). Gortázar et al. (2000) suggested three interconnected causes that would explain the clear increment of ungulates in the last decades: i) the abandonment of rural activities; ii) the subsequent decrease of livestock; and iii) the increase of forest habitats.

The increase in population density of wild boars raises concerns regarding general condition, vegetation damages and increasing prevalence of infectious diseases and parasites (Gortázar et al, 2006; Ruiz-Fons et al., 2006a). It is well known that the virulence of pathogens increases with host density (Ewald, 1993); therefore, high densities of wild boars could represent a more efficient transmission rate of pathogens and consequently an increased risk of transmission to livestock and humans. This fact should be taken into account by animal and public health authorities to promote research and epidemiological surveillance of wildlife pathogens. Wild boar densities reported in some studied populations are shown in Table 1.

Wild boar spatial ecology and social structure

Wild boars are present in a wide variety of environments across its distribution area. It can be found in flatlands, mountains, Mediterranean, continental, semi-desert and Atlantic climatic areas, and in a wide diversity of vegetation types (Abaigar, 1990). Habitat preferences of wild boars in Mediterranean habitat were observed to be oak and mixed forests (Abaigar et al., 1994). Meriggi and Sacchi (2001) also observed a great importance of forested areas for wild boars.

Age structure is mainly composed of a high percentage of wild boars less than 2 years old (Abaigar, 1990; Garzón, 1991; Fernández-Llario, 1996; Rosell, 1998) that ranges between 62 and 79 % of the population. Sex ratio does not diverge much of the 1:1 ratio between males and females in northern areas of the Iberian Peninsula (Rosell et al., 2001; Herrero, 1996), whereas higher number of females than males are consistently described throughout Europe and the rest of Spain, probably due to a biased hunting harvest (Briedermann, 1971; Abaigar, 1990; Fernández-Llario, 1996). *Suidae* have the highest reproductive rate of any ungulate family (Read and Harvey, 1989), with large

litter sizes (ranging 3.85 to 6.2 wild piglets/sow), short gestation periods and early sexual maturity.

Wild boars are gregarious animals, living in groups of variable size. Female groups with their offspring are the most frequent group pattern observed under natural conditions (Teillaud, 1986; Ahrens, 1984; Rosell et al., 2004). Females reach their sexual maturity at the age of 8 to 10 months (Rosell et al., 2001), usually when they weigh more than 30 kg. Wild boar males, under natural conditions, reach breeding age later than females (Mauget and Pepin, 1985), and yearling males usually leave maternal groups earlier than females (Rosell et al., 2001). Adult males are observed to form groups in autumn and winter, although they use to drive themselves alone during the whole year (Fernández-Llario et al., 1996; Rosell et al., 2004). Wild boar tends to spatially aggregate both due to social behaviour and to irregular food availability. Wild boar groups tend to aggregate more in autumn (Dardaillon, 1984; Rosell, 1998), probably due to diminished competence as a consequence of food resource abundance. High wild boar densities and the scarcity of water during summer months in Mediterranean countries also contribute to wild boar aggregation.

Social behaviour differences have been suggested to play a role in the epidemiology of viral diseases in wild boar such as Aujeszky´s disease (Vicente et al., 2005, **Capítulo 1.1**). Hence, pathogen transmission routes could vary within and between groups. The oronasal route could be the predominant one within groups, while, transmission between groups or from groups to individuals could be lower and partially restricted to the breeding season (venereal transmission predominantly). Thus, social structure of wild boars should be taken into account in the case of implementation of disease control programs.

The fact that wild boars are able to travel up to long distances (over 30 km, Cargnelutti et al., 1992) is epidemiologically relevant as well, since diseases may be introduced into new areas. Barriers, such as large rivers or highways, could partially limit wild boar dispersion, although wild boars would be able to pass trough barriers as reported for the Iberian wolf (Blanco et al., 2005). Fencing, in particular, is less effective for containing wild boar than for other ungulates.

Significant viral diseases in wild boar populations

Wild boar and the domestic pig share almost the same pathogens (Lipowsky, 2003). Viruses that infect the domestic pig could be found in wild boars if direct or indirect contacts between infected and susceptible animals of both swine occur. When a particular pathogen establishes a long-life cycle among a wild species, this becomes a reservoir. The absence of a wildlife reservoir for a particular pathogen is one of the most important features in order to completely eradicate the disease in the domestic population (Pastoret, 2005). Nevertheless, a disease can be controlled and eradicated in livestock despite the presence of a wildlife reservoir (Lutz et al., 2003). Since control and eradication programs are difficult to be implemented in wildlife, the remaining of a wildlife reservoir implies a risk of pathogen transmission that could become a threat for disease control and eradication campaigns implemented in domestic livestock at regional, national and international levels.

According abovementioned reasons, we considered viral diseases of importance in wild boar on the base of their direct effect among wild boars and their economic impact in domestic pig production systems.

Aujeszky's disease

Aujeszky's disease (AD) remains as one of the most important diseases of domestic pigs worldwide (included as a notifiable disease to the OIE), although many countries have successfully eradicated it in domestic pig herds (Moynagh, 1997).

AD is caused by swine alphaherpesvirus 1, also named pseudorabies virus. Domestic pig and wild boar are natural hosts of this viral infection, but the virus can also infect other mammals causing a fatal nervous disease (Pejsak and Truszczynski, 2006). Only humans and major primates seem to be resistant to AD virus (ADV) infection. ADV, as other herpesviruses, is able to establish latency in the infected host (Alemañ et al., 2001). Reactivation of latent infections and the consequent virus excretion may lead to persistence in the herd (Howarth, 1969; Davies and Beran, 1980).

Effects of ADV in wild boar populations

European wild boar and feral pig populations have been reported to be infected by ADV almost worldwide in a variable proportion (reviewed by Müller et al. 2000; Lipowsky, 2003; Lutz et al., 2003; Vengust et al., 2005; Vicente et al., 2005, **Capítulo 1.1**). In the domestic pig, ADV infection causes respiratory, reproductive and central nervous clinical signs depending on the virulence of the strain, the infective dose and the age of the host (Pejsak and Truszczynski, 2006).

Only Gortázar et al. (2002) reported an AD outbreak in wild boars from south-central Spain, where nervous clinical signs were observed both in juvenile and adult animals. Mortality was calculated for the affected population and represented 14% of the juveniles and 7% of the adults. Müller et al. (2001) observed only mild temperature increment, mild sneezing, slight nasal discharge and conjunctivitis in wild boars experimentally infected with an ADV strain of wild boar origin. However, after immunosuppressive treatment, wild boars showed severe pneumonic signs and finally died or were euthanized. Tozzini et al. (1982) observed similar signs of the infection,

although the ADV strain used was of domestic pig origin. Hahn et al. (1997) concluded that ADV strains of feral pig origin were attenuated when compared to those of domestic pigs. Obviously, new and more virulent ADV strains could have consequences in the population dynamics of wild boars, especially in dense populations.

Control of ADV in wild boar populations is also of concern for conservation purposes. The presence of ADV in wild boars can be a risk for endangered species when habitats are shared, since those species may be susceptible to ADV infection (Vicente et al., 2005, **Capítulo 1.1**).

Wild boar as ADV reservoir for the domestic pig

Since ADV is widespread in wild boar populations, their possible role as reservoir for the domestic pig has to be taken into account. Infection from the domestic pig to the wild boar and vice-versa is possible, as shown by experimental infections (Tozzini et al., 1982; Müller et al., 2001). Thus, contacts between infected and susceptible animals can lead to virus transmission. However, few reports exist regarding the risk of ADV transmission from the wild boar to the domestic pig. Müller et al. (1997) discarded the role of German wild boars as ADV reservoirs for the domestic pig on the basis of molecular differences between ADV strains of domestic pig and wild boar origin. Moreover, Germany was declared as ADV free in domestic pigs despite the virus was circulating among wild boars (Lutz et al., 2003). In a recent study (F. Ruiz-Fons et al., unpublished data, **Capítulo 4**) carried out in south-central Spain it was concluded that there was no evidence of interaction between the epidemiology of ADV in domestic pig and the wild boar.

In only one report it has been suggested that the wild boar was responsible of the appearance of AD outbreaks in open-air domestic pig herds (Hars and Rossi, 2005). Therefore, open-air domestic pig production systems represent the most risky situation

for ADV transmission but also for the transmission of other pathogens between both suids.

Classical swine fever

Classical swine fever (CSF) is caused by a *Pestivirus* closely related to bovine viral diarrhoea virus (BVDV) and border disease virus (BDV) (Wengler et al., 1995) which are also able to infect swine (Le Potier et al., 2006; Albina et al., 2000). CSF virus (CSFV) is widespread in the domestic pig, but in Western Europe and North America (Artois et al., 2002). As a highly contagious disease, it was formerly classified in the List A of the OIE, and nowadays included within the single list of diseases notifiable to the OIE. CSF is a major disease of pigs and causes high economic losses due to preventive culling of pigs, restrictions in the trade of animals in infected areas and compensation to farmers (Terpstra and de Smit, 2000).

Effects of CSFV in wild boar populations

CSFV circulates among wild boar populations in Central and Eastern Europe (Moennig et al., 1999); most of Western Europe is considered CSF-free. In those countries where the disease has been described, it is prevalent in only a limited number of areas (Artois et al., 2002).

Clinical manifestations of CSF have been found similar in wild boars and in domestic pigs after experimental infection (Depner et al., 1995; Aubert et al., 1994). Lesions are also similar in both swine, being caused generally by widespread thrombosis or endothelial damage. Moreover, susceptibility to CSFV infection is found equally for wild and domestic suids (Brugh et al., 1964; Depner et al., 1995). Acute, sub-acute and chronic clinical courses occur depending on the virulence of the CSFV strain (Brugh et al., 1964). Mortality rates also vary with the clinical course of the

disease, with higher values in acute than in sub-acute and chronic cases. High mortality rates are frequently observed in piglets both in domestic pigs and wild boars (Kern et al., 1999), especially during the onset of an outbreak.

Excretion of the virus by different ways (saliva, nasal and lachrymal secretions; Aubert et al., 1994) could lead to horizontal transmission via direct contact between infected and susceptible animals. CSFV may survive long time in protein-rich environments (Edgar et al., 1952; Helwig and Keast, 1966), which could lead to indirect transmission through carcass consumption. CSFV can also be transmitted vertically from infected sows to their foetuses during pregnancy. Transplacental transmission can lead to persistently infected animals (late-onset CSF) with no immune reaction against the virus (Meyer et al., 1980; Depner et al., 1995). Late onset infection led a wild boar piglet to death in 39 days (Depner et al., 1995), although environmental conditions to which wild boars are subjected suggest that the expected half-life for persistently infected wild boar piglets should be shorter. High mortality rates in young animals after an outbreak can lead to changes in the population dynamics of wild boars.

Wild boar as CSFV reservoir for the domestic pig

Outbreaks are generally self-limiting in wild boar populations, and after the first appearance of the outbreak, the isolation of viruses decline from several months to few years (Ferrari et al., 1998; Fritzmeier et al., 1998; Rossi et al., 2005). But in other cases, CSFV circulates for years in wild boar populations (Laddomada et al., 1994; Kern et al., 1999).

The role of the wild boar as a CSFV reservoir and possible source of infection for the domestic pig is well known. Moreover, epidemiological links between CSF virus infections in wild boar and domestic pigs have been reported repeatedly, mainly in Germany (Wachendörfer et al., 1978; Krassnig and Schuller, 1993; Laddomada et al.,

1994; Teuffert et al., 1997). Aubert et al. (1994) proposed three reasons for which wild boars should not be considered as CSFV reservoirs and risk of transmission for the domestic pig: i) when coexistence of domestic pig and wild boar is present, and CSF is eradicated from the domestic pig, the disease is not maintained in the wild boar; ii) when CSFV has been intentionally introduced in feral pig populations, the disease was not self-sustaining; and iii) when information about the origin of a CSF outbreak in wild boars was correctly collected, human interferences were evidenced. However, these considerations seem to be in contradiction with the real world: all CSF virus strains isolated from wild boars in Germany in the 1990s were also isolated in domestic pigs from the same locations; and 92% of the primary outbreaks on domestic pigs were located in regions where CSF was endemic among wild boar populations, being considered that 60% of them were due to direct or indirect contacts with wild boars (Moennig et al., 1999). Similar observations have been reported in Italy (Rutili, 1997; Ferrari et al., 1998).

The role of wild boar densities in the persistence of CSFV among wild populations after the onset of an epizootic outbreak has been suggested to have an influence together with age structure and the size of the affected population (Artois et al., 2002). It can be speculated that CSF would persist in dense wild boar populations without barrier restrictions (such as highways), due to a high recruitment rate and hence to the availability of young animals. Also, high hunting pressure in open lands could lead to yearling dispersion, and also to higher birth rates (as suggested by Rossi et al., 2005). This situation could impede control and eradication schemes of CSF due to the increased risk of transmission from wild boars to domestic pigs.

African swine fever (ASF)

African swine fever virus (ASFV) is the only member of the species *African swine fever virus*, genus *Asfivirus*, family *Asfarviridae* (Dixon et al., 2000), and is able to infect both domestic and wild suids (Sánchez-Vizcaíno, 2006). The virus is also able to replicate in soft ticks of the genus *Ornithodoros*, including *O. moubata, O. erraticus* and *O. savignyi*, from where it can be transmitted to new hosts (Sánchez Botija, 1963; Plowright et al., 1970; Mellor and Wilkinson, 1985). ASFV firstly appeared in domestic pigs in Kenia in 1921 as a consequence of transmission from wild African suids (Sánchez-Vizcaíno, 2006). The infection spread to the Iberian Peninsula, first in Portugal (Manso Ribeiro et al., 1963) and later into Spain (Polo Jover and Sánchez Botija, 1961), and since then it has appeared in several South and Central American countries and Sardinia.

ASF, like CSF, was formerly classified in the List A of the OIE, and nowadays included within the single list of diseases notifiable to the OIE. High morbidity and mortality is reported during ASF outbreaks in domestic swine, thus leading to economic losses not only due to high animal losses but also to the restrictions in trade of animals and their products. Nowadays, ASF is considered endemic in many areas of Africa, where it infects both domestic and wild swine (Sánchez-Vizcaíno, 2006). The infection is present in two wild suids from Africa, the warthog (*Phacochoerus aethiopicus*) and the bushpig (*Potamochoerus porcus*), in which the virus replicates in an unapparent form (De Tray, 1957; Heuschele and Coggins, 1965). The role of these wild suid species and *Ornithodoros* soft ticks as reservoir of ASFV for the domestic pig is largely unknown (Sánchez-Vizcaíno, 2006).

Effects of ASFV in wild boar populations

It is speculated that after the appearance of ASFV in the Iberian Peninsula, the virus spread from domestic pigs to European wild boars. Evidence of ASFV infection in

wild boars has been reported at least in Spain (Ordas et al., 1981; Pérez et al., 1998), Portugal (Da Cruz Braço-Forte, 1980) and Sardinia (Firinu and Scarano, 1988; Laddomada et al., 1993).

During the first reported ASF outbreaks, clinical signs of peracute and acute course of the disease were observed in wild boars. Later, the clinical course of the disease turned into subacute (Pérez et al., 1998). Both experimental and natural infection reports in European wild boar and feral pig agreed that macro and microscopical lesions were identical to those observed in the domestic pig (Ravaioli et al., 1967; McVicar et al., 1981).

ASFV is able to persist for at least one year in *Ornithodoros* spp. ticks (Endris et al., 1987; Hess et al., 1989). Nevertheless, Hess et al. (1989) concluded that mortality is higher in ASFV infected ticks than in non-infected ones, and suggested that this fact could be involved in ASFV clearance from tick populations that are not subjected to reinfection. To our knowledge, no reports on *O. erraticus* parasitizing wild boars are available. Nevertheless, the endophilic character of soft ticks makes it difficult to find them in hunted wild boars, the only method of tick survey applied in free-living wild boar populations.

Wild boar as ASFV reservoir for the domestic pig

No seropositive wild boars have been reported in areas where the domestic pig was free of the disease (Firinu and Scarano, 1988; Pérez et al., 1998) or, when reported, the virus circulated in very low levels among the wild population (Laddomada et al., 1993). In fact, Laddomada et al. (1994) suggested that the virus is unable to persist in wild boar populations without contact with infected domestic pigs, concluding that the role of the wild boar as an ASFV reservoir is limited.

Porcine circovirus diseases

Porcine circoviruses (PCV) are small non-enveloped, single-stranded circular DNA viruses of the family *Circoviridae*. Two PCV genotypes have been described. Porcine circovirus type 1 (PCV1), which was found as a persistent contaminant of porcine kidney cell cultures (Tischer et al., 1974), is considered non-pathogenic for swine. Porcine circovirus type 2 (PCV2) was firstly isolated from swine in Canada at the beginning of the 90s in association to a novel disease called postweaning multisystemic wasting syndrome (PMWS) (Harding, 1996). PCV2 infection in domestic swine has been further linked to other diseases or conditions including reproductive failure, porcine dermatitis and nephropathy syndrome, porcine respiratory disease complex, and others (Segalés et al., 2005). These diseases are nowadays included under the term "porcine circovirus diseases", and PMWS is considered the most significant one due to a high economic impact on the pig industry (Segalés et al., 2005).

PCV2 is worldwide distributed in domestic pig herds. Although antibodies against PCV2 have been detected in cattle (Tischer et al., 1995; Nayar et al., 1999), other studies concluded that PCV2 is not common in other ungulates but suids (Allan et al., 2000; Ellis et al., 2001; Rodríguez-Arrioja et al., 2003). The wild boar has been found not only to be infected by both PCV but also to develop PMWS both in free-living and semi-captive populations.

Effects of PCV2 in wild boar populations

PCV2 seroprevalences found in Belgian and Spanish wild boars were medium to high (30 to 40%, Sánchez et al., 2001; Vicente et al., 2004). Also, PCV2 infection has been detected in about 20% in Hungarian wild boars by PCR methods (Csàgola et al.,

2006). These reports indicate that PCV2 circulates in high rates among wild boar populations in Europe.

PMWS typically affects nursery and fattening domestic pigs (2 to 4 months of age) causing wasting, pallor of the skin, unthriftness, respiratory distress, diarrhoea and sometimes icterus (Segalés and Domingo, 2002). PCV2 is able to produce PMWS when experimental infection is carried out only with PCV2 virus (Bolin et al., 2001), although not always. Therefore, PMWS is nowadays considered a multifactorial disease in which PCV2 in necessary but not sufficient to trigger the clinical outcome (Segalés et al., 2005). In fact, other agents such as porcine reproductive and respiratory virus (PRRSV), porcine parvovirus (PPV) or *Mycoplasma hyopneumoniae* have been able to trigger PMWS in PCV2 infected pigs (Segalés et al., 2005).

Although PMWS reports in wild boars are scarce, they have been described in North America and Europe (Ellis et al., 2003; Schulze et al., 2003; Vicente et al., 2004). In all clinical cases, diseased wild boars ranged from 4 to 10 months old, except for 6 week-old farm bred feral pigs from Canada. Clinical symptoms, when they could be observed, and gross and microscopic lesions resembled those reported for the domestic pig. PMWS appearance seems to occur in older animals in wild boars when compared with domestic pig (as suggested by Vicente et al., 2004). Weaning period extends at least to 4 months of age in wild boars, in contrast with 3-4 weeks for the domestic pigs; this fact may account for the difference in age. Clinical reports of PMWS in wild boar also reported the increased piglet mortality within the herd or the hunting estate where clinical cases were found (VLA, 2003; Vicente et al., 2004).

Multiple infections are common in free-living wild boars (Ruiz-Fons et al., 2006b, **Capítulo 3**), and under immunosupressed conditions, the risk of disease development could be higher. If we take into account that other pathogens are also widely present in

European wild boar populations, PCV2 and its most significant associated disease, PMWS, could have a relevant role in mortality rates in association to other concurrent infections.

Wild boar as PCV2 reservoir for the domestic pig

At the moment, it is too premature to establish any role of wild boars as PCV2 reservoir for the domestic pig. PCV2 isolates from wild boars have been found to be identical to those from domestic pigs in the same or very distant regions (Knell et al., 2005; Cságola et al., 2006). It is very likely that the origin of PCV2 infection in wild boar populations would be due to contacts with domestic pigs, especially due to the high PCV2 infection rates (close to 100%) in pig herds. Nevertheless, there is no knowledge regarding the direction of transmission from one to the other.

Porcine parvovirosis

PPV is classified in the genus *Parvovirus* and is distributed worldwide in the domestic pig (Mengeling, 2006). All PPV isolates present similar if not identical antigenic characteristics (Johnson et al., 1976; Ruckerbauer et al., 1978). PPV has been only associated to reproductive failure in females, while acute infection of postnatal pigs is usually subclinical (reviewed by Mengeling, 2006). Thus, the major and usually only clinical effect of PPV infection is reproductive failure. Recently, PPV has been suggested to play a role as PMWS triggering in co-infection with PCV2 in domestic pigs (Choi and Chae, 2000).

Effects of PPV in wild boar populations

PPV is widely distributed in European wild boar and feral pig populations, with seroprevalences from 14 to 77% in different geographical areas worldwide (Liebermann, 1986; Payeur et al., 1989; New et al., 1994; Lutz and Wurm, 1996; Saliki

et al., 1998; Gipson et al., 1999; Roic et al., 2005; Ruiz-Fons et al., 2006b, **Capítulo 3**; Vengust et al., 2006).

Despite serologic evidence of PPV antibodies in wild boar populations, no direct effects of PPV have been described. Nevertheless, the association of PPV seroprevalence with reproductive parameters in wild boar females showed a negative influence of PPV on ovulation rate (Ruiz-Fons et al., 2006b, **Capítulo 3**). The effect of PPV on the first stages of the ovocite is unknown, but a direct effect in ovulation rate could be suggested based on these results. Reproductive failure effects depend on infection timing during gestation, thus leading to returns to estrus, resorptions, mummifications, abortion, stillbirth, neonatal death or reduced neonatal viability. When transplacental infection is produced after the second half of gestation (>70 days), foetuses are able to develop an immunologic response and to survive *in utero* (Redman et al., 1974; Bachmann et al., 1975). PPV immune animals are not able to get re-infected. Reproductive failure could be produced in the case of wild boar gilts that get pregnant for their first time without previous contact with the virus. Then, PPV infection before mid-gestation could lead to reproductive failure.

As reported above, pathogen transmission rate within female groups should be higher than between groups, thus leading to protective status of gilts within an infected group. Nevertheless, uninfected groups of females could get infected at the mating season due to contacts with infected males. Although little information is available regarding direct effects of PPV on wild boar females, the high seroprevalences found suggest that reproductive performance could be partially restricted by PPV.

Wild boar as PPV reservoir for the domestic pig

As PPV seroprevalences are higher in domestic pig herds than in European wild boar populations (with some exceptions, Lutz and Wurm, 1996), it is unlikely that the

wild boar could act as a PPV reservoir for domestic pigs. Nevertheless, transmission between both swine could take place in both ways if contacts among pigs and wild boars occur.

Porcine reproductive and respiratory syndrome (PRRS)

PRRS virus (PRRSV) is an *Arterivirus* closely related to lactate dehydrogenase-elevating virus (LDV) of mice and other viruses of the family *Arteriviridae*. It was firstly recognized in domestic pigs both in North America and Europe during early 90s (Wensvoort et al., 1991; Collins et al., 1992). Two different PRRSV genotypes are nowadays recognized, one from North America and the other from Europe, which show approximately 60% nucleotide homology (Nelsen et al., 1999). The origin of PRRSV is unclear, although a recent hypothesis has suggested that LDV infected wild boars in central Europe could be the origin (Plagemann, 2003). The author rely on the similarities of both North American and European PRRSV prototypes to LDV of mice and the introduction of European wild boars to the United States in 1912. Since then, it has been proposed that both viruses separately evolved along different evolutionary lines in North America and Europe before being spread onto domestic pigs.

PRRSV produces significant reproductive losses in domestic swine worldwide (Zimmerman et al., 2006) and is currently considered as one of the most common viral causes of porcine reproductive failure, together with PPV (Mengeling et al., 2000).

Effects of PRRSV in wild boar populations

There are limited scientific references regarding PRRSV in European wild boar and feral pig populations. Knowledge on PRRSV in wild boar populations relies on serologic results. Only 1.7% of feral pig sera tested positive in Oklahoma State, USA (Saliki et al., 1998) and 1.3% and 8.3% free-living and farmed wild boars, respectively,

in France (Albina et al., 2000). The rest of studies have yielded negative results (Oslage et al., 1994; Lutz and Wurm, 1996; Gipson et al., 1999; Vicente et al., 2002; Ruiz-Fons et al., 2006b, **Capítulo 3**; Vengust et al., 2006)

PRRSV causes a marked increase of returns to estrus, late-term abortions, stillborn and weak piglets. In many cases, severe respiratory disease in suckling and weaned pigs also occurs (reviewed by Zimmerman et al., 2006). No clinical cases of PRRS have been described in wild boars, for which clinical symptoms, if any, remain unknown. We could speculate that respiratory and reproductive disorders could occur as in the domestic pig, but the apparent low circulating rates of the virus among free-living wild boars suggest no significant influence of PRRSV in this species.

PRRSV transmission would be favoured within dense wild boar populations, but the lack of infection in many of these animal groups suggest that the initial transmission from domestic swine to wild boar does not occur or occurs very sporadically.

The suspected origin of PRRSV in European wild boars could also represent that transient evolution viruses from the original LDV infection could be present in wild boar populations and have passed unnoticed when serological tests have been used. A molecular based approach that relies on highly conserved sequences in both LDV and PRRSV would clarify this hypothesis.

Wild boar as PRRSV reservoir for the domestic pig

Currently, the transmission of PRRSV from domestic swine to wild boar is more probable than the opposite. Thus, no evidence of the wild boar as PRRSV reservoir could be suspected with the actual knowledge.

Other viral infections in wild boar populations

Other viruses have been studied in European wild boar and/or feral pig populations, such as influenza viruses, coronaviruses (transmissible gastroenteritis virus (TGEV) and porcine respiratory coronavirus (PRCV)), other pestiviruses (bovine viral diarrhoea virus (BVDV) and border disease virus (BDV)), picornaviruses (foot and mouth disease virus (FMDV), vesicular stomatitis virus (VSV) and swine vesicular disease virus (SVDV)), hepatitis E virus (HEV) and *Torque teno* viruses (TTV). We briefly review the current knowledge about these viruses in wild boar. Knowledge on viral infection status in domestic pig and wild boar as well as clinical manifestations of the reviewed diseases is summarized in tables 2 and 3.

Swine influenza

Swine influenza is caused by type A influenza viruses (Olsen et al., 2006). The domestic pig is considered as a major reservoir of H1N1 and H3N2 influenza viruses because it is the only domesticated mammal that is abundant enough and susceptible to both avian and human influenza viruses (Brown, 2000). Clinical manifestations consist in fever, cough, dyspnea and prostration that usually are followed by a rapid recovery. In domestic pig, both clinical and subclinical SIV infections are known to occur (Olsen et al., 2006). Swine are involved in the natural exchange of influenza viruses (Hinshaw et al., 1984), since swine H1N1 viruses could be introduced into bird populations (Andral et al., 1985; Ludwig et al., 1994) and H3N2 could be transmitted to humans (Kundin, 1970).

Only serologic data are available in European wild boar and feral pig populations in regard to SIV. Antibodies to three subtypes of SIV, H1N1, H3N2 and H1N2, have been detected in wild boar populations, although in variable and generally low levels (Markowska-Daniel and Pejsak, 1999; Markowska-Daniel, 2003; Markowska-Daniel and Kowalczyk, 2005). Seroprevalence may vary from 0 to 75% depending on country

or region and even SIV subtype (Saliki et al., 1998; Gipson et al., 1999; Vicente et al., 2002; Vengust et al., 2006). H1N1 subtype seems to be the most prevalent one among wild boars (Gipson et al., 1999).

Transmission of SIV is mainly produced by the oronasal route in domestic pig herds due to direct contacts between infected and susceptible animals and via aerosols. Thus, as close contacts among wild boars are density-dependent, the transmission of SIV in low-density wild boar populations will lead to the extinction of the pathogen or to very low circulating rates of the virus. Nevertheless, in semi-captive or farmed and dense wild boar populations, SIV could become endemic. Nonetheless, the scarcity of information regarding SIV status in wild boar populations only leads to speculate about the epidemiology of these viruses.

Choi et al. (2005) found that domestic pigs can get infected with high lethal Asian H5N1 viruses, although under experimental conditions these viruses are not readily transmitted. Nevertheless, the role of wild boars as possible reservoirs of the highly pathogenic avian H5N1 influenza virus should be considered.

Infection by coronaviruses

TGEV and PRCV are responsible of gastrointestinal and respiratory manifestations in domestic pigs, respectively (Saif and Sestak, 2006). TGE has been described in most countries worldwide, but its importance has decreased over time since PRCV is able to immunize pigs against TGEV infection (Saif and Sestak, 2006) and, therefore, the enzootic situation of PRCV in European domestic pigs has led to a significant decrease in the economic impact of TGE (Pensaert and Cox, 1989; Laude et al., 1993).

Little information is available regarding coronavirus infections in European wild boars and feral pigs. Feral pigs investigated in the USA did not show antibodies against

TGEV (Woods et al., 1990; Saliki et al., 1998). Moreover, no TGEV antibodies were found in Slovenian wild boars (Vengust et al., 2006), although 3% of the analysed wild boars had anti-PRCV antibodies. Although more information is needed, the available data suggest that coronaviruses infections are not common among wild boar populations and, therefore, wild boars are not expected to act as a reservoir for the domestic swine.

Infection by other pestiviruses

BVDV and BDV infect a wide variety of domestic and wild ungulates (Nettleton, 1990; Depner et al., 1991; Vannier and Albina, 1999). Both agents are classified in the genus *Pestivirus* together with CSFV. BVDV and BDV are morphologically and structurally indistinguishable (Laude, 1979).

Serologic evidence of both infections has been reported in wild boar populations (Dahle et al., 1993; Albina et al., 2000). New et al. (1994) did not find BVDV antibodies in feral pigs from the USA. No other information is available for both pathogens in wild boar populations. A role of wild boars as BVDV and BDV reservoir is not expected with the available data. Nevertheless, serological cross-reactions with CSFV false positives have to be taken into account when surveys are carried out.

Infection by picornaviruses

FMD is a disease that affects wild and domestic ungulates (Thomson et al., 2001). It is a highly contagious disease with a high impact in the trade of animals and their products, and it was formerly classified in the List A of the OIE and nowadays included within the single list of diseases notifiable to the OIE.

FMD had been widely reported in domestic animals in Europe and currently persists in the northern part of South America, most African countries, the Middle East, and some countries of Eastern Europe and in Asia (Thomson et al., 2001). In contrast,

VSV is endemic in the American continent (Lubroth et al., 2006). Both VSV an SVDV are important as a differential diagnosis with FMDV infection due to similar clinical signs (Lubroth et al., 2006).

The domestic pig and also the wild boar are natural hosts for these picornaviruses. Information is scarce in relation to these viral diseases in wild boar populations. Nevertheless, Pech and Hone (1988) estimated as highly important the possible role of feral pigs if a FMD outbreak would enter to Australia. Thus, wildlife is to be considered in regard to FMD.

Hepatitis E

HEV is a single, non-enveloped, positive-stranded RNA virus belonging to the *Hepeviridae* family (Emerson et al., 2004). Hepatitis E is an important disease of public health concern due to its zoonotic character. HEV has been widely reported infecting domestic swine herds around the world (Clayson et al., 1995; Meng et al., 1997, 1999; Chandler et al., 1999; Hsieh et al., 1999; Pina et al., 2000).

HEV transmission from wild animals to humans has been reported due to consumption of raw or under-cooked deer or wild boar meat (Matsuda et al., 2003; Tei et al., 2003; Li et al., 2005). HEV has been found in wild boar both by serology and molecular analyses (Takahashi et al., 2004; Nakamura et al., 2006). Nevertheless, the scarcity of knowledge of the sanitary status of wild boar populations in regard to HEV outside Japan makes it impossible to establish both the impact of the disease among wild boars and the possible role of the wild boar as HEV reservoir for the domestic pig. However, the relative widespread HEV infection in domestic pigs in Europe suggests that it would be quite probable to find HEV in European wild boar populations.

Infection by Torque teno viruses

TTV was firstly detected from a human patient with post-transfusion hepatitis of unknown etiology (Nishizawa et al., 1997). TTV was later isolated from other domestic animals such as pigs, cats, dogs, cattle, sheep and chicken (Leary et al., 1999; Okamoto et al., 2002), although the virus is considered species-specific. This virus has been reported to be present in almost 100% of the domestic pig herds in many different countries (McKeown et al., 2004). Recently, two different genogroups of swine TTV have been identified (Niel et al., 2005), and both are highly prevalent in the domestic swine (Kekarainen et al., 2006). TTV is nowadays considered non-pathogenic for all the species where it has been found, but swine TTV genogroup 2 has been found more prevalent in PMWS affected pigs than in non-affected pigs (Kekarainen et al., 2006). Therefore, further studies are required to assess the potential role of this virus in disease or disease triggering.

Only one survey is available on the presence of TTV in wild boars and one or the other swine TTV genogroups were found in 84% of the tested animals (Martínez et al., 2006). Moreover, this study found differences regarding management, age, sex and TTV genogroup, but their significance remains to be assessed.

Discussion

Viral diseases represent a threat for production efficiency in industrialized pig producing countries as well as a great impact in those underdeveloped countries where pig meat is an important food resource. Viruses infecting domestic pigs are also able to infect the wild boar. These facts, together with the increasing economic relevance of wild boars for the emerging hunting industry led us to review knowledge on viral diseases affecting wild boars under two points of view: their effect on wild boar population dynamics and the reservoir role of the wild boar for the domestic pig.

Diseases are suffered by wildlife species in a similar manner than domestic animals do. Many wildlife species are able to get infected with a pathogen that is also able to infect domestic animals or humans, and then it can become a reservoir. Three points have to be taken into account in order to consider an animal species as a reservoir: 1) It must be abundant; 2) It must be able to get infected by the pathogen; and 3) It must be able to transmit the agent to other animals (Wobeser, 1994; Corner, 2006). On that score, the wild boar could act as reservoir of viruses for the domestic pig, as has been evidenced in the case of CSFV in Central Europe.

Some of the reviewed viral diseases are able to produce great impact on wild boar population dynamics, especially those coursing with high mortality rates. Other diseases should actuate at a more subtle level, modelling survival or reproductive rates. Nevertheless, there is still not enough information about the impact of viral and other infectious diseases on wild boar dynamics. Moreover, there is a lack of information regarding pathogenesis, clinical manifestations, epidemiology and prevention and control methods of viral diseases in wild boars. Although both the domestic pig and the wild boar are considered as the same species and basic features of the viral infection could be identical, risk factors of disease widely differ between domestic and wild species. This knowledge needs to be improved.

Several characteristics make the wild boar a very interesting species for epidemiological research on wildlife diseases: 1) Its worldwide distribution; 2) The fact of sharing common infectious and parasitic agents with the domestic pig; 3) Its great ability to adapt to different environments and to colonize new habitats; 4) Its great reproductive rate and thus its ability to recover from population declines; 5) Its complex social behaviour; and 6) Its good adaptation to captivity and hence the possibility of controlled experimental research. The wild boar is able to maintain some viral

pathogens without the intervention of domestic or other wild animals. Thus, it is a true reservoir of several viral pathogens for the domestic pig. Avoiding close contacts between wild boar and domestic animals would be of importance for disease control and eradication programmes. Moreover, as artificial feeding and other management methods employed for hunting purposes could lead to density increase and higher contact rates among wild boars, promoting more natural conditions of wild boar management could lead to a decrease in infectious disease prevalences.

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Table 1. Wild boar densities across its distribution areas according to reported scientific literature.

Country/region	**Wild boar/Km^2**	**References**
Aragón (Spain)	2.8-4.2	Herrero et al. (1995)
Burgos (Spain)	1.9-4.2	Tellería and Saez-Royuela (1986)
León (Spain)	1.7-11.4	Purroy et al. (1988)
Cataluña (Spain)	3.6-8.5	Rosell (1998)
Extremadura (Spain)	3	Garzón (1991)
Castilla-La Mancha (Spain)	1.2-90.9	Acevedo et al. (2006b)
France	1-2.9	Dardaillon (1984); Spitz et al. (1984)
Italy	1.4-1.7	Marsan et al. (1995)
Byelorussia	1.8	Okarma (1995)
Poland	3.5	Jedrzejewski et al. (1997)
Germany	5.6	Reported by Howells and Edwards-Jones (1997)
Russia	1.2-1.9	Reported by Howells and Edwards-Jones (1997)
California (USA)	5.8	Reported by Howells and Edwards-Jones (1997)

Table 2. Viral pathogen status in domestic pig and wild boar populations. The role of wild boar as reservoir is reported.

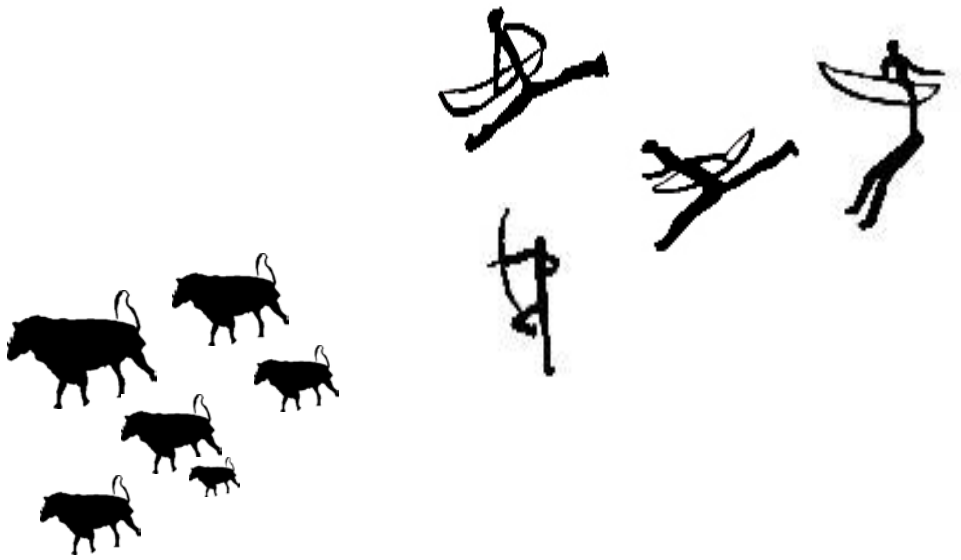
Pathogen	Domestic pig status	Wild boar status	Wild boar reservoir role for the domestic pig
ADV	Worldwide spread. Eradicated in many northern European countries and part of North America. Under eradication in Mediterranean countries	Widespread. Low prevalences in central Europe. Medium-high prevalences in Mediterranean countries and feral pig populations from USA	Discarded in Germany and in a Spanish region at large scale. Local cases due to contacts caused by inadequate pig restriction methods
CSFV	Present in many central and eastern European countries. Absent in most of Western Europe	Prevalent in a limited number of areas in different Central and Eastern European countries	Self-limiting infection in some populations and self-maintained infection in others. Persistence of the infection associated to population size, reproductive rate and density
ASFV	Present in most of Africa	Eradication reported in many American countries and the Iberian peninsula	Reported as self-limiting infection in the absence of infected domestic pigs.
PCV2	Widespread in domestic pig herds worldwide	Only reported in Europe and Canada, with medium seroprevalences in Belgium and Spain	Unexpected due to high prevalences in domestic pig herds
PPV	Widespread in domestic pigs with very high prevalences	Medium-high seroprevalences in European wild boar and feral pig	Unexpected due to high prevalences in domestic pigs
PRRSV	Worldwide present in domestic pig herds	Only serological evidence in wild boars from France and, maybe, USA	Unexpected
SIV	Considered a major reservoir of H1N1, H1N2 and H3N2. Able to get experimentally infected by high lethal H5N1 virus	Serologic evidence of H1N1 virus in feral pigs and in European wild boar from Spain. Serologic evidence of H1N1, H3N2 and H1N2 in European wild boars from Poland	Unknown
TGEV & PRCV	PRCV widely present in domestic pig herds worldwide; TGEV present worldwide but sporadically	Limited information. TGEV absent in wild boar. Three percent PRCV seroprevalence in Slovenian wild boars	Unexpected under the actual status
BVDV & BDV	Sporadical and mainly due to contacts or shared habitat with domestic ruminants	BVDV antibodies reported only in France as differential diagnosis with CSFV antibodies	Unexpected under the actual status
FMD	Enzootic in most areas of Africa, Asia and South America.	Without evidence	Unexpected
VSV & VSDV	VSV endemic in North America. VSDV present in European domestic pig herds	Unknown	Unknown
HEV	Worldwide distributed	Serologic and molecular evidence in Japanese wild boars	Unknown. Possible source for humans due to raw or uncooked wild boar meat consumption
TTV	Widespread in domestic pigs	Widely present in Spanish wild boar populations	Unknown

Table 3. Main clinical signs of viral diseases in domestic pigs and wild boars.

	Clinical manifestation in domestic pigs	Clinical evidences in wild boars
ADV	Dependent on age, infective dose and strain virulence. Nervous (piglets), respiratory (mainly in growing) and reproductive (sows) manifestations	Only evidence of nervous signs in naturally infected animals. Severe respiratory signs after immunosuppressive treatment of experimentally infected animals
CSFV	Depending on the clinical course of the infection; more severe in acute than in chronic course. Anorexia, fever, conjunctivitis, constipation, diarrhoea, hyperaemia of the skin, posterior paresis, purplish discoloration in abdomen, snout, ears and medial sides of the legs, convulsions	Clinical signs similar to domestic pig. High mortality rates in young wild boars
ASFV	Severe haemorrhagic disease in all age classes	Clinical course identical to domestic pigs
PCV2	Cause of PMWS: wasting, unthriftness, pallor of the skin, respiratory distress, diarrhoea, and occasionally icterus. PCV2 is also implicated in other porcine circovirus diseases	Few reports of PMWS affected farmed and free-living wild boars, with same clinical signs than domestic pig. Unknown if other PCVD occur in wild boars
PPV	Reproductive failure in females. Associated to PMWS triggering in some cases	Associated to lower ovulation rate. Expected to be similar than that in domestic pigs
PRRSV	Respiratory and reproductive signs. Associated to PMWS triggering in some cases	Unknown
SIV	Fever, cough, dyspnea and prostration, generally of rapid recovery	Unknown
TGEV	Transient vomiting, yellowish diarrhoea, weight loss, dehydratation	Unknown
PRCV	Respiratory signs of severity dependent on strain. Severity improved in co-infection with PRRSV	Unknown
BVDV & BDV	Commonly sub-clinic	Unknown
FMD, VSV & VSDV	Clinical signs cannot be distinguished between FMD, VSV & VSDV. They consist in fever, formation of vesicles and erosions in snout, lips, tongue, hard and soft palate and coronary band of the feet	Unknown
HEV	No clinical signs reported. Slight hepatic inflammation only seen histopathologically	Unknown
TTV	Currently considered as non-pathogenic	Unknown

Capítulo 2

Distribución y situación del virus de la enfermedad de Aujeszky en las poblaciones de jabalí de la España peninsular



Capítulo 2.1

Estudio serológico del virus de la enfermedad de Aujeszky en el jabalí en España

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Serosurvey of Aujeszky's disease virus infection in European wild boar in Spain

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Resumen

Se analizaron muestras de suero de 693 jabalíes (*Sus scrofa*) por medio de técnica ELISA de bloqueo, y la prevalencia media (se) de anticuerpos contra del virus de la enfermedad de Aujeszky fue del 44 (4) por ciento. Todos los jabalíes seropositivos procedían del centro-sur de España, excepto uno del centro de España, cercano a la principal zona positiva. En esta zona, donde las especies de caza mayor son cada vez más manejadas con fines cinegéticos, la seroprevalencia se vio afectada por el tipo de manejo. Las poblaciones con mayor intensidad de manejo presentaron mayor prevalencia que los jabalíes en situaciones más naturales, aumentando la seroprevalencia con la edad; la seroprevalencia fue mayor en las hembras de todos los grupos de edad. La seroprevalencia en los machos de más de un año de edad presentó un pico tras la época de celo, mientras que las hembras de la misma edad presentaron un mayor y más constante nivel de seroprevalencia a lo largo del año.

Abstract

Serum samples from 693 hunted wild boars (*Sus scrofa*) were analysed by means of a blocking ELISA technique, and the mean (se) prevalence of antibodies to Aujeszky's disease virus was 44 (4) per cent. All the seropositive wild boars were from south central Spain, except for one from central Spain, close to the main positive area. In this area, where large game species are increasingly managed for hunting, the seroprevalence was affected by the type of management. More intensively managed populations had a higher prevalence than wild boar living in natural situations, and the seroprevalence increased with the age of the animals; the seroprevalence was higher in females in all age groups. The seroprevalence in males more than one year old peaked

after the breeding season, whereas females of the same age had a higher and constant seroprevalence throughout the year.

Introduction

Aujeszky's disease virus (ADV) or pseudorabies virus is an alphaherpesvirus that infects the domestic pig, feral pig and wild boar (*Sus scrofa*) as natural hosts and also a wide range of domestic and wild mammals (Kluge et al., 1999). The disease occurs worldwide and causes heavy economic losses owing to its direct effect on domestic pigs and its indirect impact on the international trade in pig products. It is recognised as an important disease by the Office International des Epizooties (OIE) and is included on its List B. Most European countries, including Spain, are carrying out eradication programmes, and disease-free areas have already been established (Moynagh, 1997). In areas where the prevalence of the virus among domestic pigs is high, wild boar must be taken into account in the control schemes. However, Germany has recently become ADV-free despite the relatively high ADV seroprevalence among free-living wild boar (Lutz et al., 2003).

The virus causes a fatal infection in other species, including carnivores such as the Florida panther (*Felis concolor corii*) and the European brown bear (*Ursus arctos*) (Glass et al., 1994; Zanin et al., 1997). In Spain, endangered carnivores such as the Iberian lynx (*Lynx pardinus*), the brown bear and the Iberian wolf (*Canis lupus signatus*) may include wild boar among their prey species (Valverde, 1967; Cuesta et al., 1991; Clevenger et al., 1992), and thus may also be at risk of ADV infection.

The pathogenicity of Aujeszky's disease in pigs depends on their age, immunological and reproductive status, the strain of the virus, and the infective dose (Kluge et al., 1999). The virus is transmitted mainly by air flow, as an aerosol, and under favourable conditions can travel up to 80 km (Christensen et al., 1993). Venereal

transmission may also be important in the epidemiology of the disease in feral pigs (Romero et al., 2001).

The European wild boar is the most widely distributed ungulate on the Spanish mainland, and its range and population density have increased during the past three decades (Saez-Royuela and Tellería, 1986; Gortázar et al., 2000). Owing to its abundance, it is also one of the most popular game species. In order to increase the hunting harvest, wild boar populations are managed by high-wire fencing, artificial feeding and restocking with farm-bred individuals. As a result, some wild boar hunting estates resemble extensive pig-breeding facilities, with high population densities, but with almost no sanitary care. These changes in wildlife management have already given rise to concerns about the control of infectious and parasitic diseases (Gortázar et al., 2002; Fernández-de-Mera et al., 2003; Parra et al., 2003), and they may also affect the epidemiology of Aujeszky's disease. A clear example of the relationship between the type of management and the spread of porcine circovirus type 2 among wild boar populations has been described by Vicente et al. (2004).

Outbreaks of Aujeszky's disease have rarely been described in wild boar, and only one outbreak has so far been reported in Spain (Gortázar et al., 2002). In that outbreak, the course of the disease was acute and mortality affected mainly young individuals. In contrast, there is extensive literature concerning the seroprevalence of ADV in wild boar or feral pigs worldwide (Müller et al., 2000) and a few reports describing the isolation of the virus (Capua et al., 1997). Evidence of latently infected wild boar has been described by Capua et al. (1997) and Müller et al. (1998). The European wild boar may constitute a wildlife reservoir of ADV (Lipowski, 2003). Molecular approaches to ADV epidemiology in wild boar have suggested that strains

from free-living wild boar are different from those isolated from domestic pigs, but that they may have a common origin (Capua et al., 1997; Müller et al., 2001).

The aims of this study were to investigate the distribution of ADV seroprevalence in wild boar populations in Spain, and the role of individual, seasonal and management-related factors in any variations in its distribution. Because venereal transmission may be important (Romero et al., 2001), the relationship between the reproductive phenology of the wild boar and ADV seroprevalence was also studied.

Material and methods

Sampling sites and period

Between 2000 and 2003, samples of serum were collected from 693 hunted wild boar in 56 Spanish localities (Fig 1); most of them were collected in the main hunting season (October to February), but 25 were collected in summer, when some hunting is allowed to limit crop damage. The sampling sites were representative of the most relevant types of landscape on mainland Spain, with a bias towards the Mediterranean shrublands of the central and southern regions, where hunting activities are most important (Fernández-Llario et al., 2003). The sampling sites were divided into six regions, Asturias, Ebro Bassin, Guadiana, Jaén, Montes de Toledo and Sierra Morena (Table 1). More precise descriptions of these areas have been given by Vicente et al. (2004).

At each sampling site, the management conditions to which the wild boar were subjected were recorded as either open (230 samples from 22 sites), fenced (374 samples from 29 sites), or intensively managed areas with fencing, translocations and artificial feeding (89 samples from five sites).

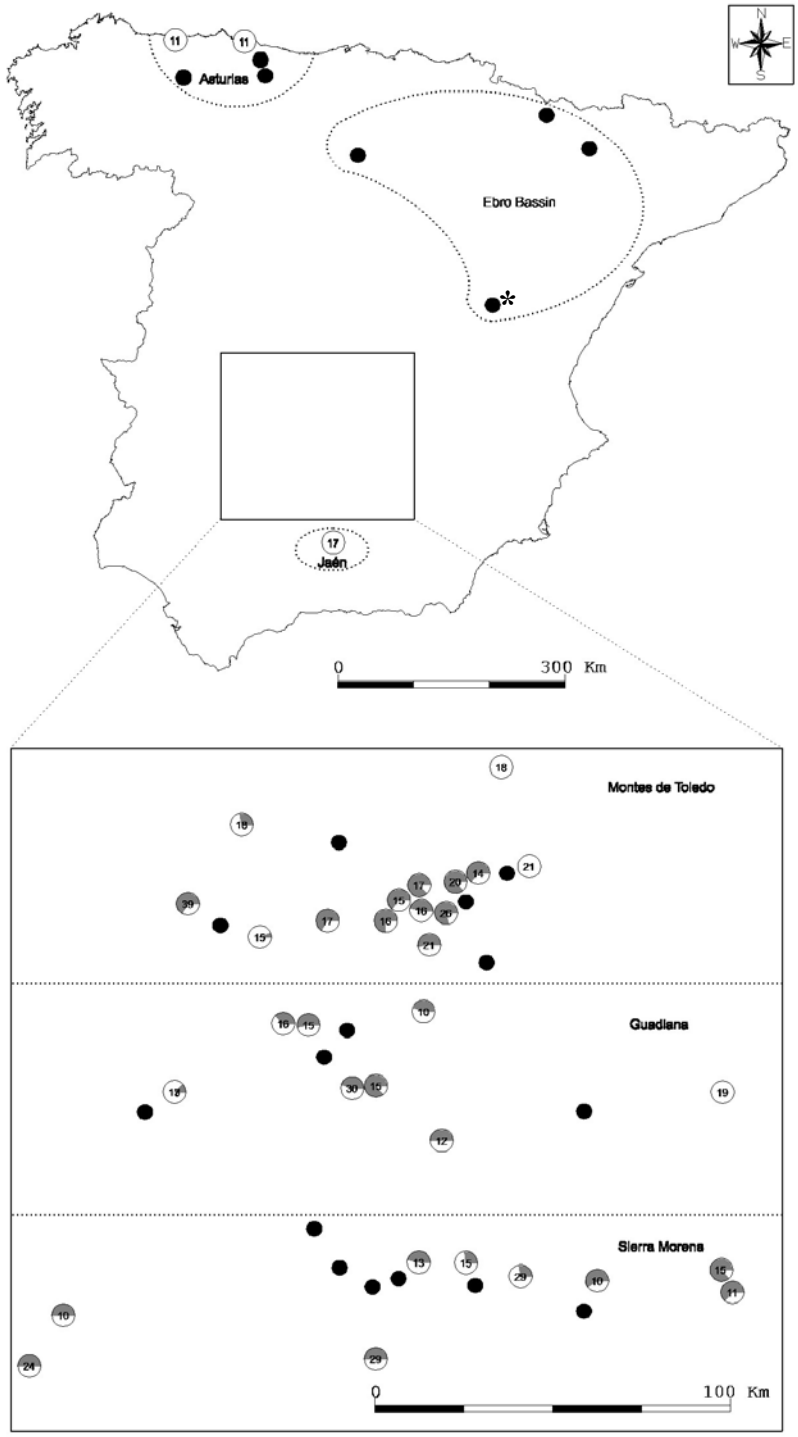

Figure 1: Map of the Spanish mainland showing the sampling sites, the number of samples analysed from each site and the prevalence of samples with antibodies to Aujeszky's disease virus (the percentage of grey colour in relation to the whole circle). Dots represent sampling sites where fewer than 10 samples were obtained. * Location of a single seropositive sample

Interviews and estimates of abundance

Twenty-eight of the sampling sites were visited in September 2002, immediately before the hunting season, to obtain information about the local game management and to estimate the abundance of wild boar on the basis of counts of droppings. No estimates of abundance were made on the sites sampled after September 2002. Through a personal interview, information was obtained from gamekeepers about the presence or absence of fencing, artificial feeding and any translocations of wild boar on the estate. A dropping frequency index was used to estimate the relative abundance of the wild boar, as described by Vicente et al. (2004).

Table 1: Number of sampling sites, number of samples analysed, and the prevalence of samples seropositive to Aujeszky's disease virus, with 95 per cent confidence intervals (CI), in the six regions of Spain sampled.

Area	Nº sites	N	Nº Positive	Prevalence (%)	1,96 S. E. (95% C.I.)
Asturias	5	30	0	0	0
Ebro Bassin	4	20	1	5	10
Guadiana	12	146	59	40	8
Jaén	1	17	0	0	0
M. Toledo	19	286	151	53	6
S. Morena	15	194	95	49	7
Total	56	693	306	44	4

Field data

Each of the 693 wild boar was measured and its sex determined; 378 female and 294 males were identified, but the sex of the other 21 was not recorded. On the basis of their tooth eruption patterns, animals between seven and 12 months old were classified as juveniles, those between 12 and 24 months as subadults, and those over two years as

adults (Saenz de Buruaga et al., 1991). Blood samples were collected from the heart during postmortem examination and serum was obtained by centrifugation and stored at –20°C until used. To study the relationship between the reproductive phenology of the wild boar populations and the seroprevalence of ADV, the reproductive tracts were collected from the females and the time at which conception had occurred was calculated using the Huggett and Widdas formula from the average weight of the fetuses (Fernández-Llario and Mateos-Quesada, 1998).

Blocking ELISA

The serum samples were analysed by using a commercial ELISA (Chekit Aujeszky-ELISA; Bommeli Diagnostics) following the manufacturer's recommendations. The test is a blocking ELISA based on the detection of specific anti-gE antibodies and has previously been used in wild boar (Dahle et al., 1993; Müller et al., 1998, 2001; Albina et al., 2000).

Statistics

Ninety-five per cent confidence intervals (CI) for the standard errors (se) of the prevalence (p) were estimated from the expression $S.E._{95\%C.I.} = 1.96[p(1-p)]/n^{1/2}$ (Martin et al., 1987). Because all but one of the ADV-seropositive animals came from areas in south central Spain (Montes de Toledo, Guadiana and Sierra Morena), the animals from these areas were used for statistical purposes. To test for significant differences in the seroprevalence, comparisons were made between boar of different sexes and age groups, and under different systems of management, and their interactions, by using a log-linear analysis, reporting the partial chi-squared values derived from a saturated model. The ages and management systems were treated as categorical variables with three classes as described above. An analysis of variance was used to study the effect of

the management system on the population density of the boar. The level of significance was established at 5 per cent. The SPSS 10.0.6 program was used for the statistical analyses.

Results

Antibodies to ADV were found in 306 of 693 samples (mean [se] 44 [4] per cent), and the prevalence differed among the geographical regions as shown in Table 1. Fig 1 shows the percentage of seropositive wild boar at each sampling site. Except for one individual from the Ebro Bassin region, all the seropositive animals were from south central Spain, the core area. In this region, only three of 31 sites at which at least 10 animals had been sampled had no seropositive boar. There were significant differences between the estimates of abundance of wild boar at the different sampling sites, depending on their management system ($P<0.001$), and the seroprevalence of ADV also differed significantly between sites with different management systems ($P<0.001$). The highest prevalences were observed in intensively managed populations, and the lowest in open populations. Juvenile boar from the intensively managed hunting estates had a much higher proportion of seropositive animals than juveniles from fenced or open populations (Fig 2).

There were significant differences between the age classes ($P<0.001$) (Fig 3), with higher seroprevalences in older animals; females of all age classes had higher prevalence rates than males ($P<0.001$) (Fig 3). The oestrus period was seasonally restricted and peaked in October and November. There were significant differences between the sexes in the prevalence of ADV in February, but the differences decreased to a minimum just after the breeding season in December (Fig 4), mainly as a result of an increase in prevalence among males, whereas the prevalence among females remained constant throughout the year.

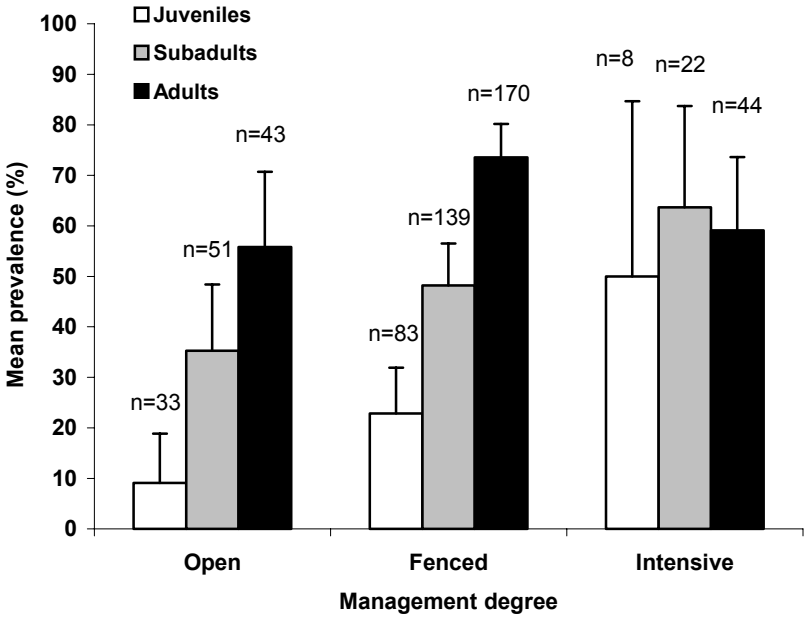

Figure 2: Percentages (95 per cent confidence intervals) of 593 wild boar with antibodies to Aujeszky's disease virus, depending on their age and on the management system of the site in south central Spain from which the samples were taken.

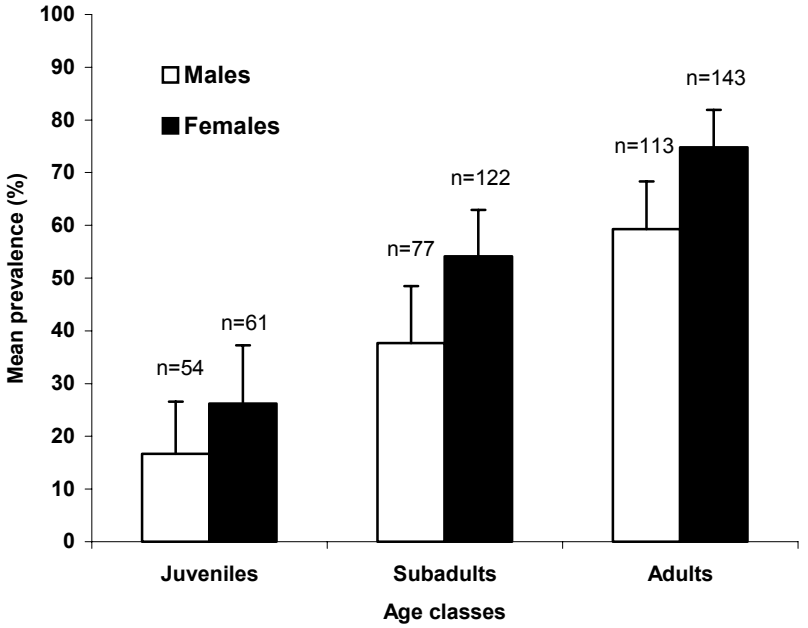

Figure 3: Percentages (95 per cent confidence intervals) of 570 wild boar from south central Spain with antibodies to Aujeszky's disease virus, depending on their age and sex.

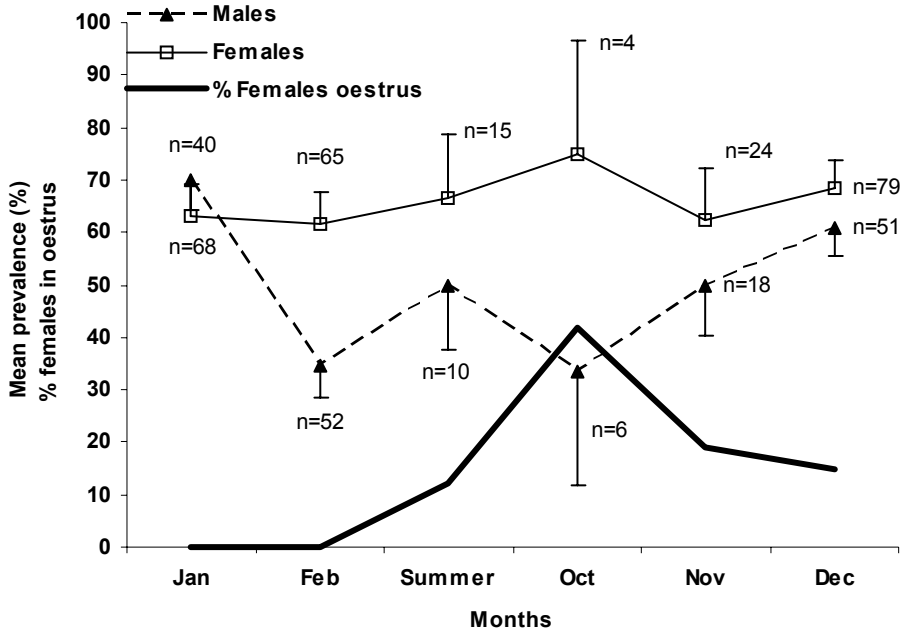

Figure 4: Mean (se) prevalences of 177 male and 255 female wild boar with antibodies to Aujeszky's disease virus at intervals during the year. The prevalences were significantly different in February.

Discussion

These results suggest that ADV is widespread among populations of wild boar in south central Spain, and the prevalence of antibodies to ADV reported from other European populations of wild boar is usually lower than those found in Spain. For example, the mean prevalences were between 0 and 14 per cent in mainland France, 0.6 and 23.5 per cent in Germany, and 0 and 41.8 per cent in Italy (Albina et al., 2000; Müller et al., 2000; Lutz et al., 2003); however, the prevalence on the island of Corsica was higher (61.5 per cent) (Albina et al., 2000). The prevalences in south central Spain varied across the different estates, with a maximum of 93 per cent at one site (14 of 15 samples positive). The prevalence in the core area was close to the level reported in feral pigs in the USA (Van der Leek et al., 1993; Müller et al., 2000; Gresham et al., 2002), and similar to previous findings from the same and neighbouring regions in Spain (Vicente et al., 2002). However, the sampling effort was inadequate in some

regions reported to have high population densities of wild boar, such as the Pyrenees. More samples from north-west, north-east and eastern Spain, and from the south and south west, would be desirable.

Management factors may explain the different seroprevalences found at the different sampling sites. The population density of wild boar is high in the core area, but low in most parts of northern Spain (Gortázar et al., 2000, 2002). This difference, together with management factors that may also affect the transmission of the disease, such as fencing and artificial feeding, may explain why only one seropositive wild boar was found outside the core area. Translocations of wild boar are increasingly common in Spain, and the risk of introducing ADV or other disease agents into disease-free areas should be of concern.

The high prevalence of ADV among Spanish wild boar populations may have consequences for these animals, and for other wildlife and domestic livestock. Although outbreaks of ADV have been reported among wild boar only occasionally (Gortázar et al., 2002), the effect of the virus may be more subtle, affecting the animals' reproduction and the survival of piglets. These less evident effects on wild boar population dynamics will be analysed in more detail in a future study, taking into consideration other infections, such as porcine parvovirus, that can also affect reproduction. As stated in a study of porcine circovirus type 2 (Vicente et al., 2004), a lack of information on the seroprevalence in piglets makes the study of the effect of any disease on wild boar population dynamics difficult.

Domestic pigs are apparently infected by different strains of ADV than those that infect wild boar (Müller et al., 2001), but there is still the risk of an eventual reinfection of regions declared free of ADV (Moynagh, 1977; Lutz et al., 2003). In general terms, seropositivity to ADV is high in domestic pigs in Spain (Gutiérrez-Martín et al., 2000).

The presence of the virus in wild boar could interfere with the programmes to eradicate ADV from Spain, especially in extensively bred Iberian pigs. Molecular epidemiology, based on viral isolation and sequencing, is needed to determine whether any strains are shared between wildlife and domestic animals, and to obtain knowledge to improve the current eradication schemes.

Conservation issues are also of concern in relation to ADV in Spanish wildlife. The core area, where the seroprevalence of ADV in wild boar is highest, includes Sierra Morena, which is one of the last two strongholds of the Iberian lynx, considered the most endangered felid in the world (Nowell and Jakson, 1996). The recent decline in its main prey species, the European wild rabbit (*Oryctolagus cuniculus*), as a result of a viral disease (Villafuerte et al., 1994), favours the consumption of alternative prey, including wild boar (Valverde, 1967). Since cases of Aujeszky's disease have already been reported among felids (Glass et al., 1994), the risk of lynxes dying as a result of contact with infected wild boar cannot be excluded. It has been suggested anecdotally that hunting dogs dying after consuming wild boar meat may have been infected with ADV.

Two previous studies have also observed a higher seroprevalence of ADV among female wild boar (Jridi et al., 1996; Lutz et al., 2003); Lutz et al. (2003) did not determine the cause of this finding. In the present study the difference may be explained by two factors; first, the difference in age at which male and female boar reach sexual maturity: under natural conditions, males reach breeding age later than females (Mauget and Pepin, 1985), which may start breeding at six to eight months (Rosell et al., 2001). Secondly, yearling males usually leave their maternal group and thus have fewer intraspecific contacts except during the breeding season (Rosell et al., 2001). The infection levels in males only became comparable with those in females

immediately after the breeding season. The lower levels of seroprevalence among males during the rest of the year may be due to the incorporation of seronegative young male animals into that age class (over one year). The authors suggest that venereal transmission may be important during the breeding season for males, whereas in females, which already have higher prevalences than males during the breeding season, social gregariousness may favour direct routes of infection, such as the respiratory route.

Intensive game management, which increases the population density and probably the number of intraspecific contacts, together with the social organisation of the wild boar, may contribute to the observed pattern of seroprevalence. Different management structures could have associated risk factors. Baiting and supplementary feeding, mainly practised in fenced enclosures, could increase the rate of transmission of ADV and affect its prevalence in a particular herd. In order to control ADV in wild boar in Spain, it would be desirable to promote the more natural way of managing them, as is practised in the north of the country, rather than the intensive, livestock-like management system that is used in many parts of south central Spain.

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Capítulo 2.2

Patrones de infección del virus de la enfermedad de Aujeszky en el jabalí

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Aujeszky´s disease virus infection patterns in European wild boar

Enviado a: ***Veterinary Microbiology***

Resumen

La evidencia de exposición (mediante seroprevalencia) al virus de la enfermedad de Aujeszky (ADV) es elevada en los jabalíes del centro-sur de España. Este trabajo pretende determinar la presencia del ADV por medio de técnicas moleculares, así como describir los patrones de infección por el ADV en los jabalíes. Se recogieron tonsilas (TN) y ganglios trigéminos (TG) para detección molecular y sueros de jabalíes (n=192) procedentes de 39 fincas de caza del centro-sur de España (2004/2005). Se realizó reacción en cadena de la polimerasa anidada (PCR) para un fragmento de la glicoproteína de membrana B del ADV en los tejidos recogidos. El estado individual de presencia de ADN del virus se analizó con respecto a variables exploratorias por medio de Modelos Generalizados Lineales Mixtos (GLIMMIX). La prevalencia vírica fue del 30.6 ± 6.7%. A pesar de que se observó un patrón de incremento con la edad y de que las hembras presentaron mayores seroprevalencias, no se observó influencia estadísticamente significativa del sexo y la edad para la presencia vírica. Los resultados moleculares en TN y TG permitieron clasificar el estado de infección en cuatro clases: negativo (sin ADN del ADV ni en TN ni en TG), presencia de ADN solamente en TN, en TN y TG, y solamente en TG. Se encontraron diferencias estadísticamente significativas del estado de infección por el ADV en los animales con ADN del virus en TN con respecto al sexo. Observamos que todos los jabalíes con ADN del ADV en TN y TG y solamente en TG, excepto uno, reaccionaron positivamente en el ELISA. En contraste, animales con ADN del virus solamente en TN reaccionaron serológicamente tanto positiva como negativamente. Interesantemente, el 45% de los jabalíes positivos en la PCR (n=59) fueron seronegativos en el análisis serológico, todos ellos con presencia de ADN del virus solamente en TN. Este resultado es de crucial relevancia desde el momento en que los sistemas actuales de manejo en nuestro estudio promueven

el traslado de animales con fines cinegéticos, con el riesgo asociado de no detectar individuos infectados por el ADV cuando se usa la serología para chequear la infección por el ADV.

Abstract

Evidence of exposure (i. e. seroprevalence) to Aujeszky´s disease virus (ADV) is high among wild boars from south-central Spain. This research aims to determine the presence of ADV by molecular detection, and to describe the patterns of ADV infection in wild boars. Tonsils (TN) and trigeminal ganglia (TG) for ADV molecular detection, and sera were collected from wild boars (n=192) in 39 hunting estates from south-central Spain (2004/2005). A nested polymerase chain reaction (PCR) for a fragment of the ADV surface glycoprotein B was performed on collected tissues. Individual status of presence of viral DNA was tested against explanatory variables by means of a Generalized Linear Mixed Model (GLIMMIX) analysis. Viral detection prevalence was 30.6 ± 6.7%. Although there was an increasing pattern with age and females presented higher prevalences, no statistically significant influence of sex and age was found for viral presence. Molecular testing in TN and TG allowed classifying infection status into four classes: negative (no ADV DNA in TN and TG), viral DNA presence only in TN, in TN and TG and only in TG. Statistically significant differences of ADV infection status were found only for animals with ADV DNA in TN when plotted against sex. We observed that all but one wild boar with ADV DNA both in TN and TG and only in TG reacted positive in the ELISA. In contrast, animals with only ADV DNA in TN serorreacted positively and negatively. Interestingly, 45% of the PCR positive wild boars (n=59) were seronegative in the serological test, all of them with viral DNA only in TN. This is of great concern since current management schemes in our study promote

animal translocation for hunting purposes, with the associated risk of under-detecting ADV infected individuals when using serology to screen for ADV infection.

Keywords: Infection; Polymerase chain reaction; Pseudorabies; Risk assessment; Serology.

Introduction

Aujeszky´s disease virus (ADV), or pseudorabies virus, is a worldwide distributed swine alphaherpesvirus that infects wild and domestic swine as natural hosts (Kluge et al., 1999). ADV also infects a wide range of other hosts except humans and major primates (ibid.). Mammals other than swine are considered dead-end hosts because infection is fatal before virus excretion. ADV has the ability of establishing a lifelong latent infection in neuronal and non-neuronal cells of its natural hosts (Alemañ et al., 2001). This particularity of herpesviruses can lead to virus persistence at herd level due to the reactivation of latent infections and consequent virus excretion (Howarth, 1969; Davies and Beran, 1980). This feature remains one of the most important issues regarding ADV epidemiology in the domestic pig (Rock, 1993) and the wild boar or feral pig (Lutz et al., 2003; Romero et al., 2003).

ADV is a highly neurotropic virus, and after the primary entrance to the host the virus first replicates in the nasopharyngeal mucosa, tonsils and the olfactory epithelia (Kit, 1999). ADV invades the central nervous system (CNS) through the nerve ends in the tonsils and the upper respiratory tract (Wittman et al., 1980). Highly virulent strains are able to extend to the rest of the CNS, where they produce a nonsuppurative meningoencephalitis that causes fatal disease in piglets (Card and Enquist, 1995). Nervous clinical signs as a consequence of nonsuppurative meningoencephalitis have also been described in wild boars (Gortázar et al., 2002). Low virulent strains do not massively invade the CNS and instead establish a lifelong latent infection in neuronal

cells near the point of entrance (Vannier, 1986). Cell-associated viremia has been reported after experimental infection of domestic pigs (Nauwynck and Pensaert, 1995). Nevertheless, the presence of the virus in mononuclear cells in peripheral blood is not consistent in the case of latent infections (Balasch et al., 1998). Although the presence of ADV latency associated transcript has been detected in tonsils of latently infected pigs (Cheung, 1995), trigeminal ganglia (TG) are considered the major site for ADV latency (Gutekunst et al., 1980; Brockmeier et al., 1993; Tham et al., 1994). ADV was rarely detected by PCR in tonsils of latently infected pigs (Balasch et al., 1998).

Concerning the European wild boar, after experimental infection with a German wild boar strain, ADV was detected in TN, lungs, spinal cord and pons (Müller et al., 2001). Lutz et al., (2003) detected ADV genome in TN of naturally infected wild boars. None of these studies analysed TG from wild boars. Naturally infected feral swine showed the presence of ADV mainly in the sacral ganglia (56%) and TG (44%) (Romero et al., 2003). Transmission of ADV in feral pigs can occur by the respiratory route, by infected carcasses consumption (Hahn et al., 1997) and the venereal route (Romero et al., 2001). Strains of feral pig origin seem to be attenuated, as experimental infection did not cause clinical signs and nearly no humoral response (Hahn et al., 1997). ADV strains of wild boar origin also seem to be of low virulence (Tozzini et al., 1997; Müller et al., 2001), although immunosuppressive treatment led to respiratory signs in experimentally infected wild boars with an ADV strain of wild boar origin (Müller et al., 2001).

Wild boar populations have shown a tendency to increase through the last decades in south central Spain (Gortázar et al., 2006), among other factors due to the development of a commercial hunting industry (Acevedo et al., 2006). Seroprevalence (an evidence of exposure) of ADV is high among these populations (Vicente et al. 2005,

Capítulo 1.1). Recently even fatal outbreaks have been observed (Gortázar et al., 2002), which is not surprising since pathogen virulence potentially increases with increased host population density (Ewald, 1993). Given the special nature of ADV infections, where latent infected animals play an important role in disease spread, a more epidemiological precise knowledge based in molecular evidence is needed. This research aims to determine the presence of ADV by molecular detection, and how ADV detection correlates with serological results.

Materials and methods

Sampling sites

Sampling of wild boars was conducted in 39 public and private hunting estates located in the South Central Spanish Plateau, with the exception of few hunting estates located further south (n=1) or north (n=3) (see Figure 1). The South Central Spanish Plateau is a flat region devoted to agriculture surrounded by medium-high mountainous elevations, crossed east-to-west by Toledo Mountains (MT). The climate of the study region is continental Mediterranean, where annual rainfall ranges from 300 to 700 mm concentrated in spring and autumn.

Through a personal interview to game keepers, we obtained data regarding management (artificial feeding and watering, fencing, sanitary measures, translocations among others). Hunting estates were open (no fencing with low degree of management) or fenced (fencing and usually artificial feeding) (see Vicente et al., 2004).

Wild boar and hunting estate data

Hunter harvested wild boars (n=192) were sampled during the hunting season 2004/2005 (sampling is biased to the period from October to February). Sex (n=59 males and n=128 females), age and total length were recorded. Sampled wild boars

were classified in two different age classes according to teeth eruption pattern (Saenz de Buruaga et al., 1991). Wild boars less than 24 months old were classified as juveniles (n=97) and those over 24 months as adults (n=86). Individual body condition was assessed by the Kidney fat index (Vicente et al., 2005. **Capítulo 1.1**). Blood was collected directly from the heart into sterile tubes, maintained refrigerated until arrival at the laboratory, and after centrifugation, sera were frozen at -30ºC in 1.5ml aliquots. During necropsy, tonsils (TN) and trigeminal ganglia (TG) were collected, identified and separately transported under cool conditions to the laboratory. TN and TG were separately stored at -80ºC.

Serological analyses

Wild boar sera (n=185) were tested for the presence of glycoprotein E (gE) antibodies by means of a commercial blocking ELISA (Checkit-PRVgI, Bommelli Diagnostics, Switzerland). This serologic test is based on whole ADV antigen coated plates. Sensitivity and specificity for domestic pigs reach values of 95.2-98.9% and 97.8-99.5%, respectively (according to manufacturer's data). Serologic values obtained from the sampled animals were grouped in 10% stepped classes, ranging from -80 to 120. The cut off of the test is fixed in a serologic value of 55%, according to results obtained by manufacturer from domestic pigs. Samples below this value are considered as negative, those between 55 and 65% as doubtful and positive above 65%.

DNA preparation

DNA from TN and TG was extracted using a commercial DNA extraction kit (Nucleospin®Tissue, Macherey-Nagel, Germany). Twenty-five mg of each sample were used for DNA extraction. DNA aliquots obtained were frozen at -30ºC until PCR performance (for no more than 7 days before the PCR was performed). Also, DNA was

extracted from a gE deleted attenuated vaccine strain (Porcilis Begonia, Intervet, The Netherlands), and from a north-eastern Spanish domestic pig isolated virulent strain (Juanola strain, provided by Dr. E. Mateu) by means of commercial DNA extraction kit (Nucleospin®Blood, Macherey-Nagel, Germany). We consistently used these ADV samples and sterilized bi-distilled water throughout the analytical process as extraction and PCR positive and negative controls, respectively.

Polymerase chain reaction

Five µl of extracted DNA were used to perform a nested PCR for an ADV glycoprotein B (gB) fragment amplification as previously described (Mengeling et al., 1992; Balasch et al., 1998). As highly conserved genes in ADV are suggested to be used for PCR purposes (Müller et al., 2000), we chose gB as a highly conserved glycoprotein across ADV strains. The gB gene of ADV was targeted for nested PCR amplification using forward primer 5´-ATGGCCATCTCGCGGTGC-3´ and reverse primer 5´-ACTCGCGGTCCTCCAGCA-3´ at the first stage (Mengeling et al., 1992). A product of 334 bp was obtained after the first stage amplification. Second stage amplified a product of 195 bp using forward primer 5´-ACGGCACGGGCGTGATC-3´ and reverse primer 5´-GGTTCAGGGTACCCCGC-3´. Polymerase chain reactions were performed in 50 µl reaction volumes in both stages with primer concentration of 0.2 µM, 200 µM dNTP´s and 1 U of TAQ polymerase per reaction vessel (Biotools, B&M Labs, Madrid, Spain). The first stage consisted in 20 cycles of 95ºC for 1 min of denaturation, 60ºC for 45 s of annealing and 72ºC for 30 s of elongation. The second stage consisted in denaturation at 95ºC for 1 min, annealing at 65ºC for 45 s and elongation at 72ºC for 30 s in 30 cycles. PCR reactions were performed in GeneAmp PCR System 2700 thermocycler (Applied Biosystems, Foster city, USA). Finally, 20 µl of the amplification reaction were resolved by electrophoresis in 2% agarose gels. DNA

amplified bands were visualized by ultraviolet transillumination after staining with ethidium bromide.

Wild boar that reacted negatively in both TN and TG were classified as negative; those ADV positive were classified either as only TN positive, only TG positive, or positive in both TN and TG. We calculated the frequency of wild boars (n=129) grouped in each of the 10% stepped serologic values according to their ADV infection status.

PCR detection limit

In order to test for the detection limit of the gB nested PCR, we made serial 1/10 dilutions until $1/10^5$ using Juanola strain at 10^4 $TCID_{50}$/ml (quantified by means of a viral neutralization assay). We used 200 µl of the Juanola strain solution alone or mixed with 25 mg of non-ADV infected wild boar tonsil as undiluted samples. The limit detection of the used nested PCR was calculated as the least dilution at which a 195 bp band was evidenced by ultraviolet transillumination in a 2% agarose gel.

Statistical analyses

The factors affecting the risk of an individual to test positive to ADV presence by PCR (categorical, negative *vs.* infected) was tested by means of a logistic analysis (n=129) (Generalized Linear Mixed Models, GLIMMIX). Variables included in the analyses as explanatory were estate type (categorical, open *vs.* fenced), sex (categorical, male *vs.* female), age class (categorical, juvenile and adult), total length (as continuous) and Kidney fat index (% as continuous, Vicente et al., 2005, **Capítulo 1.1**). Starting from univariate models, a multivariate analysis was performed following a forward procedure according to the Akaike Information Criterion (AIC) (Burnham and Anderson, 1992). To control for local and regional effects, hunting estate and

geographic area of sampling were considered as random categorical variables. We fitted the models with a logistic link function and a binomial error. Confidence intervals for standard errors of prevalence were estimated with the expression $S.E._{95\%C.I.} = 1.96[p(1-p)]/n^{1/2}$ (Martin et al., 1987). χ^2 tests were performed to assess statistical differences between prevalences and seroprevalences.

Results

Serial dilutions of the 10^4 $TCID_{50}$ Juanola strain with and without ADV-free wild boar tonsil resulted in a limit detection of the nested PCR of 10 $TCID_{50}$/ml and 10^{-2} $TCID_{50}$/ml, respectively.

Mean prevalence of ADV viral presence by PCR was 30.6 ± 6.7% in the sampled wild boars. We did not find sex or age related statistically significant differences (Table 1, Figure 2) and none of the other tested variables showed a statistically significant influence on the individual risk of ADV infection (Table 1, the final model explained the 33% of the dependent variable total variance and showed an AIC=589.3). Prevalence of viral presence was 33.6 ± 11.2% for females and 24.1 ± 8.4% for males. Concerning age classes, prevalence was 33.7 ± 10% for adults and 27.6 ± 9% for juveniles.

Out of the 192 PCR analysed wild boars, 133 (69.3 ± 9.3%) were negative for the presence of the virus both in TN and TG, 46 (24 ± 11.2%) had presence of viral DNA only in TN, 6 only in TG (3.1 ± 14.9%), and 7 (3.6 ± 13.7%) had ADV DNA both in TN and TG. Prevalences of viral presence in TN and in TN and TG across sex and age are shown in figure 3. We found that the 78% of the infected animals (n=59) had viral DNA only in TN (10% and 18% had ADV DNA only in TG and both in TN and TG, respectively).

Females had more frequently ADV DNA in TN than males (χ^2=6.19, p<0.05; 26.2 ± 11.4 and 17.2 ± 15.5% for females and males, respectively), but not in the case of viral DNA presence only in TG (χ^2=1.35, p=0.2; 8.2 ± 11.4 and 6.9 ± 15.5% for females and males, respectively). There was no significant age-related difference in the proportion of infected individuals with viral material only in TN (χ^2=0.05, p=0.5) and only in TG (χ^2=1.35, p=0.2).

Table 1: Final model obtained with wild boar individual variables and estate type tested for their influence on the individual risk of testing positive for Aujeszky´s disease virus presence. Degrees of freedom (DF), F value and *p* are shown.

Variable	**Num DF/ Den DF**	**F**	***p***
Kidney fat index	1/108	2.22	0.14
Age class	1/112	0.96	0.33
Sex	1/108	0.79	0.37
Total length	1/117	0.00	0.94

Out of the 185 analysed sera, 45.9 ± 7.8% had antibodies against ADV gE. Seroprevalence was also higher in females (50 ± 8.8%) than in males (35.7 ± 12.7%), and these differences were statistically significant (χ^2=11.51, p<0.001). Also, statistically significant differences were evidenced regarding seroprevalence across age (χ^2=8.85, p<0.05), with more positive adults (65.1 ± 10.4%) than juveniles (21.6 ± 8.6%).

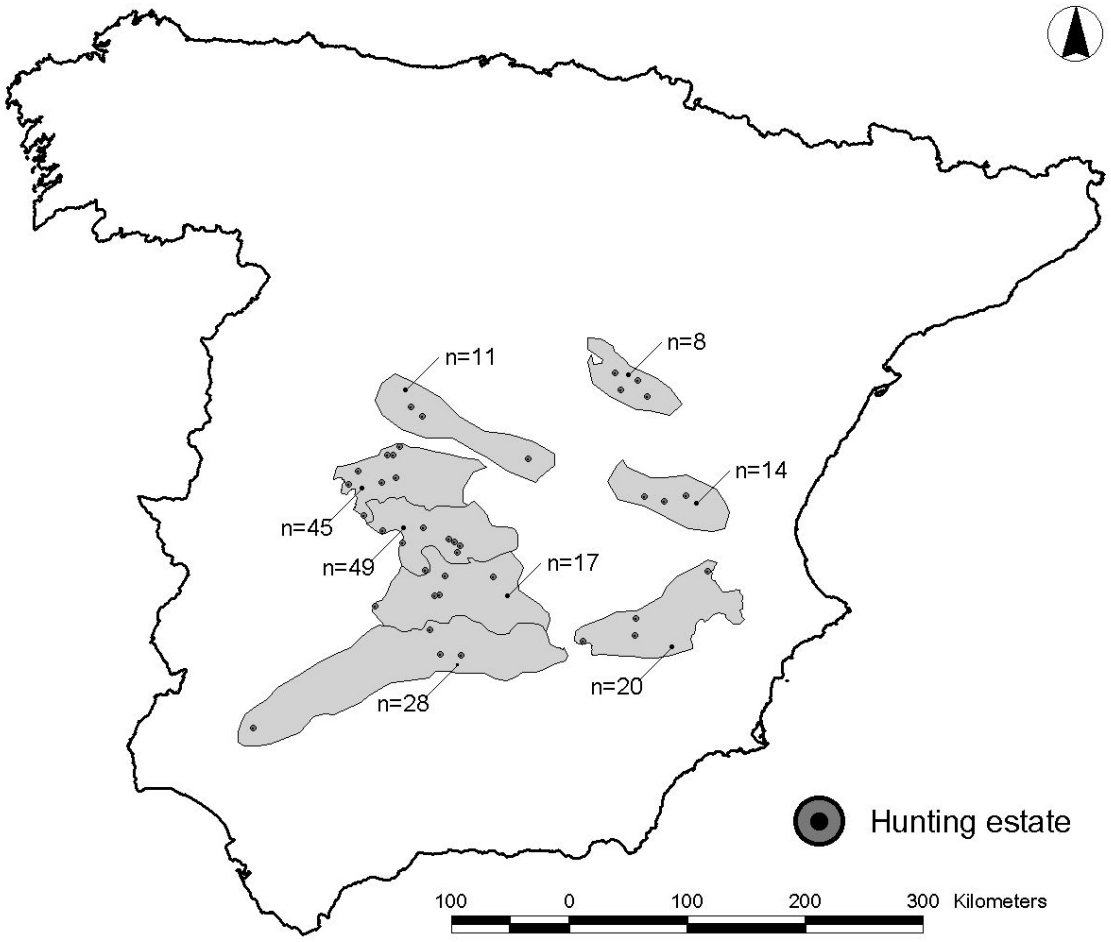

Figure 1. Sampled hunting estates in the different geographic areas considered in the epidemiological analysis. The number of sampled wild boars per geographic area is shown.

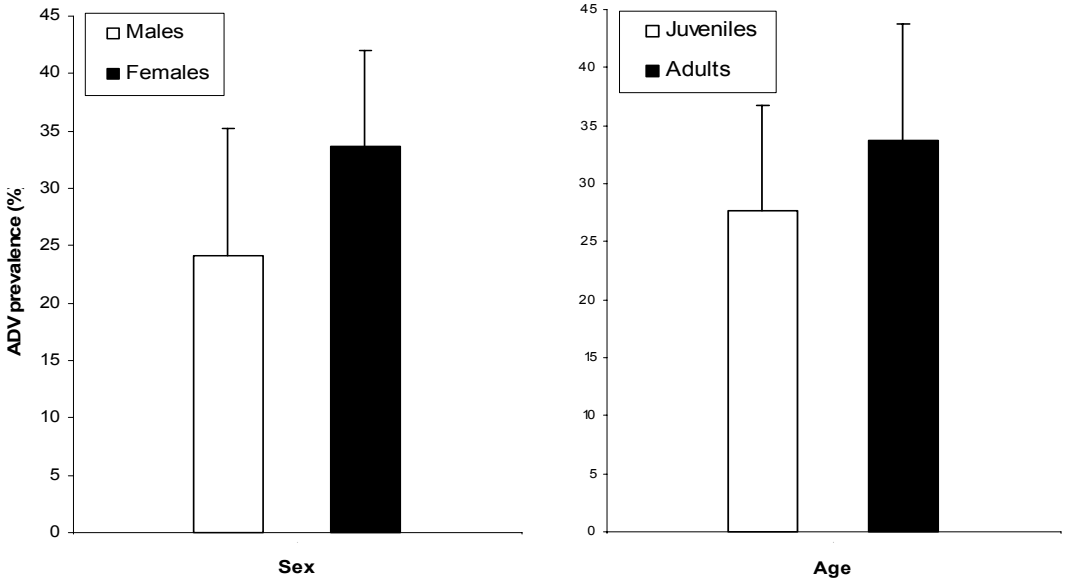

Figure 2: Aujeszky´s disease virus prevalence (by PCR test) across sex and age class. No statistically significant differences were observed between sex and age class, as shown in table 1.

All the wild boars with ADV DNA only in TG and in both TN and TG (n=12) showed inhibition values above the cut off point (Figure 4), thus being considered seropositive, except one that tested doubtful. In the case of animals with viral DNA only in TN (n=46), 26 (56.5%) showed inhibition values below the cut off point (seronegative) and 16 (34.8%) above it (seropositive). Forty-five percent of the PCR positive animals reacted negative in the serologic test. PCR negative wild boars (n=75) showed both, inhibition values above (53.3%, n=40) and below (46.7%, n=35) the cut off point. As a consequence, it was statistically significantly more probable to test seropositive for individuals with ADV DNA in TG and in both TN and TG than for those only positive in TN (χ^2=10.70, p<0.01) or negative to ADV infection (χ^2=10.08, p<0.01), whereas infected individuals only in TN were not more prone to test seropositive than individuals negative to ADV infection (χ^2=0.19, p<0.67).

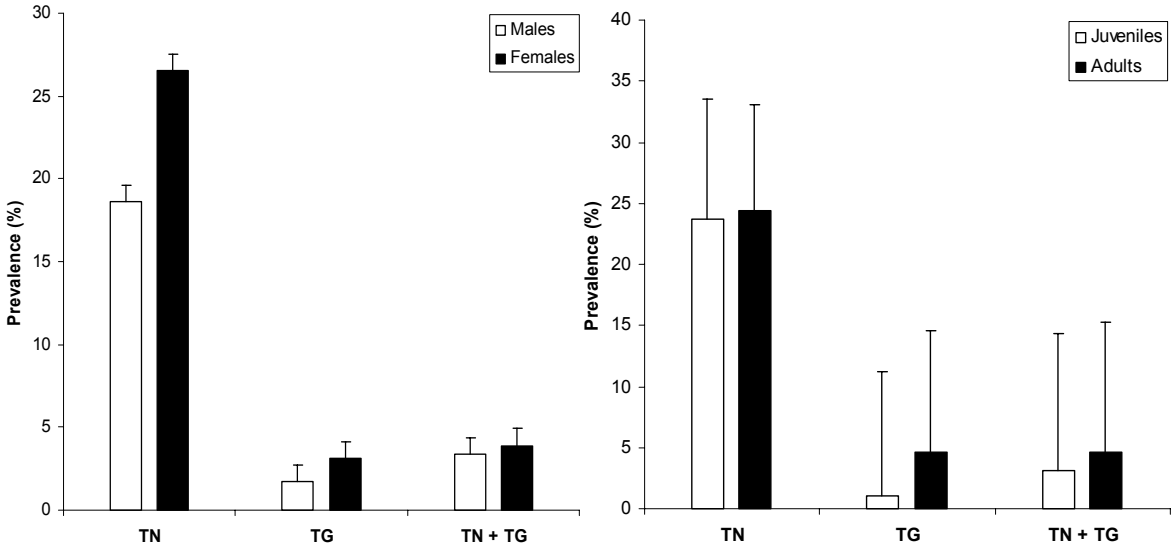

Figure 3: Aujeszky´s disease virus infection status (ADV DNA only in TN, only in TG and in both TN and TG) across sex and age classes. Prevalences of infection only in TN statistically differed between both sexes (χ2=6.19, p<0.05).

Discussion

Previous studies confirm that ADV antibodies circulate in a high percentage of the south-central Spanish wild boar populations (Vicente et al., 2005, **Capítulo 1.1**). Here, we show that a large proportion of animals carry viral DNA including a remarkable number of infected individuals positive to ADV DNA in TN that test negative upon serology. This finding is of concern since current management schemes in our study area that promote animal translocation for hunting purposes rely on serological testing, with the associated risk of under-detecting ADV infected individuals. We also found that a high number of seropositive wild boars tested negative by PCR. In the following, we provide a discussion of the probable mechanisms determining the seroprevalence and infection patterns that we found, and the sanitary concerns raised by this research.

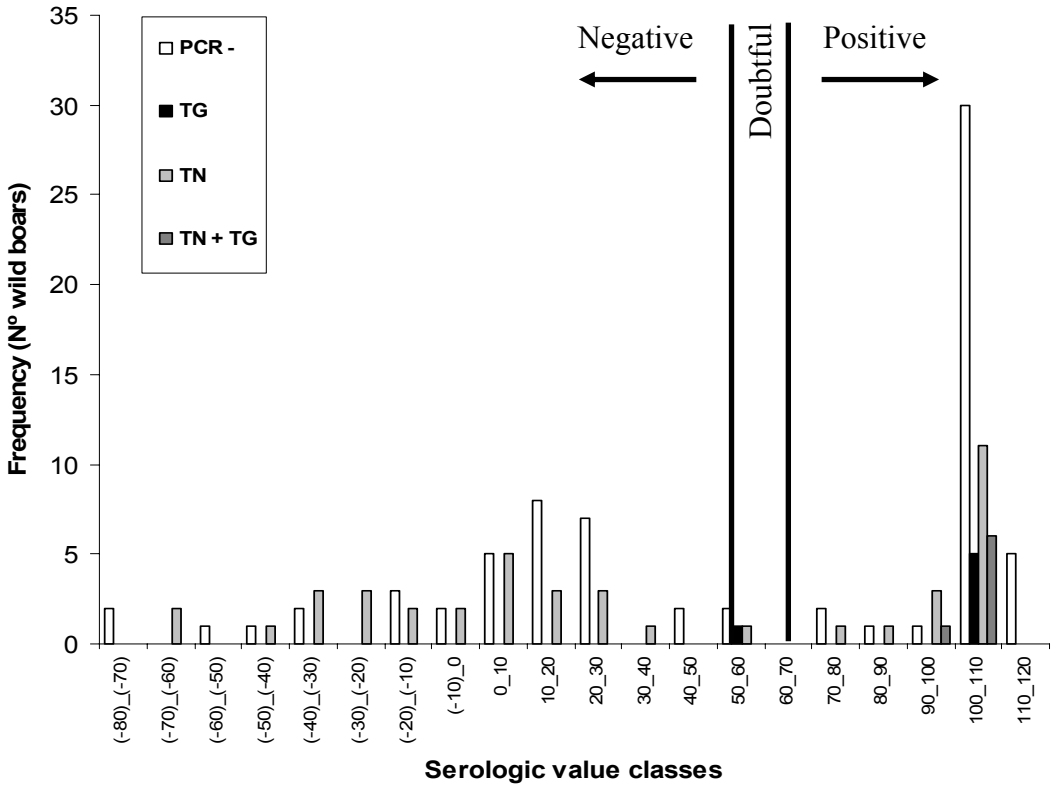

Figure 3: Blocking ELISA serologic values (10% stepped classes) of the sampled wild boars plotted against individual ADV infection status (PCR negative, ADV DNA only in TN, only in TG and both in TN and TG). Inhibition serologic values fewer than 55% are considered negative, between 55 and 65% doubtful and over 65% positive.

ADV can be detected in TN and nasopharyngeal epithelium directly after infection. Then, through the nerve ends, ADV invades the CNS and is detected both in TN and TG. After replication, the appearance of neutralizing antibodies leads ADV to establish a latent infection, commonly in neuronal cells of the TG. In this case, the virus is rarely detected in TN (Balasch et al., 1998). After immunosuppressive treatments or stress conditions, ADV can reactivate and replicate outside the sites of latency (Tanaka and Mannen, 2003), being detectable both in TN and TG. As only TN and TG were considered in the present study, it was impossible to differentiate between primary infections where the virus had arrived to TG and reactivated infections, where the virus had spread from TG to other tissues. Nevertheless, serologic results for wild boars with ADV DNA both in TN and TG showed the presence of detectable antibody titres in serum.

The nested PCR used in our study has been previously employed in order to detect ADV in latently infected domestic pigs (Balasch et al., 1998), being considered as very useful for this purposes. It has proven to have a very sensitive detection limit, thus enabling us to be confident to have detected most of the animals that carried ADV. The fact that detection of viral material does not allow to decide if there is viable virus present may limit the meaning of our results somewhat, but PCR has been employed previously for the diagnosis of individual status of ADV infection and is considered a more sensitive technique than viral isolation (Müller et al., 2000). In general, PCR has proven to be very useful for the detection of low levels of genomic sequences of several different viruses (Laure et al., 1988; Murakawa et al., 1998) and makes the amplification of rare DNA sequences by a factor of 10^5 to 10^6 possible (Maes et al., 1990).

We found that a large proportion of the infected animals (78 %) had viral DNA only in TN, which could indicate a high contact rate between animals. Lutz et al. (2003) found that only piglet and juvenile wild boars showed the presence of ADV in TN. Although no piglets were sampled in this study, we found juvenile and adult animals being infected. A moderate force of ADV infection in our study populations would allow for recent infections occurring at all age classes, taking in account that the number of infected animals only in TN did not differ between juvenile and adult wild boars (Figure 3).

Previous sero-epizootiologic studies by others authors report higher ADV seroprevalences in females than in males (Lutz et al., 2003; Jridi et al., 1996). This is consistent with our findings and could be due to different social behavioural traits and/or to age differences in sexual maturing between males and females (Vicente et al., 2005, **Capítulo 1.1**). As close contacts between females and offspring are increased within the group, ADV transmission at within-maternal group level could be predominantly oral or respiratory. Contacts between females and males occur in the mating season (i.e. autumn, see Vicente et al., 2005, **Capítulo 1.1**), which may involve reactivation of latent virus due to mating stress and subsequent venereal transmission (Romero et al., 2001). Both the respiratory/oral and the venereal routes of infection could be of importance for the European wild boar (Lutz et al., 2003), whereas for the feral pig, experimental infection suggested that venereal route is more important (Romero et al., 2001, 2003). As females showed higher values of infection only in TN than males (26.2% and 17.2%, respectively), a higher transmission rate among females could exist and may relate to sexual contacts to males, which are polygynous, and also to higher aggregation and contact rates. In spite of an apparent increase in seroprevalence with age, we did not find any difference in ADV infection across age

classes. The increase in seroprevalence with age has been associated to increased probability of exposure to ADV (Lutz et al., 2003; Vicente et al., 2005, **Capítulo 1.1**).

Wild boars with ADV DNA both in TN and TG showed seropositive (one doubtful) results when sera were tested, which indicates that anti-gE antibodies circulate among these wild boars in detectable levels for the used test. Interestingly, not all wild boars with viral DNA detected by PCR in TN showed detectable levels of anti-gE antibodies. Previous authors report detection of ADV specific antibodies at 15 and 26 days after contact with experimentally infected wild boars with an ADV strain of wild boar origin (Müller et al., 2001). Moreover, Romero et al. (1997) reported the fact that four of five contact feral pig females did not show the presence of low levels of neutralizing antibodies until 8 weeks after contact with naturally infected males. Lutz et al. (2003) isolated ADV from seronegative naturally infected wild boars. They also reported that only 3 of 13 PCR positive wild boars had ADV-specific antibodies when tested with two different ELISA tests. Thus, the appearance of detectable levels of antibodies in peripheral blood could be delayed in time after infection with ADV strains of wild boar origin. The use of serologic tests in order to determine ADV status at an individual level in wild boars should consider that recently infected animals without detectable levels of antibodies could be missed. This is of concern in regard to wild boar translocations.

Fifty-three percent of the PCR negative animals reacted positive in the serologic test. Based on the specificity of the serologic test, we could expect that only 0.4 to 1.6 of the 75 PCR negative wild boars would react as positive. It is unknown if wild boars are able to efficiently eliminate ADV after infection. We could hypothesize that this result could be explained by cases of venereal transmission of ADV strains that remain latent in nervous cells of sacral ganglia, or by lack of specificity of the employed ELISA

test when wild boar sera are tested. The suspected attenuated character of wild boar ADV strains could suppose latent infection only in sacral ganglia if ADV transmission is produced by the venereal route. The restriction to nervous sites near the route of entrance has been suggested for attenuated ADV strains (Vannier, 1986). Thus, we could be underestimating the real infection rate in the sampled wild boars. Romero et al. (2003) could not find ADV DNA in 4 of 17 naturally infected feral swine despite being able to detect ADV-specific neutralizing antibodies. Experimental infection of wild boars and molecular evidence of ADV presence in different tissues depending on the way of virus entrance would be needed in order to clarify these results.

The results of our study show that ADV infection is widespread in south-central Spanish wild boar populations, as predicted already by high seroprevalence levels. This is especially relevant for wild boar health status, for conservation issues (as ADV could infect endangered mammals such as the Iberian lynx *Lynx pardinus*) and for veterinary authorities with regards to AD control and eradication campaigns. We also found that the oral/nasal route is important in the transmission of ADV among wild boars. Finally, determining individual ADV status in wild boars by serology only appears to have an associated risk of missing recently infected animals without detectable circulating antibody levels. As mentioned above, this is of great concern in regard to wild boar translocation purposes into ADV-free areas. Also latency of ADV is of concern, as the stress related to capture, handling and transport could reactivate a latent infection causing the animals to shed infectious virus. Further research should be carried out in order to clarify the main routes of infection and the pathogenesis of ADV in wild boars, both in experimental and field studies.

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Capítulo 3

Seroprevalencia de seis patógenos reproductivos en jabalí (*Sus scrofa*) en España: el efecto sobre la función reproductora en las hembras de jabalí

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Seroprevalence of six reproductive pathogens in European wild boar (Sus scrofa) from Spain: The effect on wild boar female reproductive performance

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Resumen

Estudiamos la seroprevalencia de seis patógenos reproductivos en hembras de jabalí cazadas en España. La muestra fue representativa de la caza en las fincas estudiadas. La prevalencia media de anticuerpos fue: 60.6 ± 0.06% para el virus de la enfermedad de Aujeszky (ADV), 56.6 ± 0.09% para el parvovirus porcino (PPV), 51.8 ± 0.06% para el circovirus porcino tipo 2 (PCV2), 29.7 ± 0.09% para *Brucella* spp. y 36.3 ± 0.1% para *Toxoplasma gondii*. No detectamos anticuerpos contra el virus del síndrome reproductivo y respiratorio porcino (PRRSV). La seroprevalencia de ADV se asoció con las de PPV y PCV2 en las hembras de jabalí españolas. La tasa de ovulación en las hembras de jabalí estudiadas fue 4.41 ± 0.16 (n=120), el tamaño medio de camada fue 3.91 ± 0.16 (n=82) y el índice de reabsorción parcial fue 0.92 ± 0.17 (n=66). La tasa de ovulación y el tamaño de camada se asociaron estadísticamente a la edad. La seroprevalencia de *T. gondii* se relacionó negativamente con la tasa de ovulación y el índice de reabsorción parcial. Los jabalíes de fincas manejadas presentaron anticuerpos contra más patógenos que los de fincas abiertas. Las relaciones potenciales entre el manejo de las poblaciones de jabalíes y la exposición de los individuos a diferentes patógenos reproductivos son discutidas.

Abstract

We studied the seroprevalence of six reproductive pathogens in Spanish hunter-harvested wild boar females. The sample was representative of the hunting harvest in the studied hunting estates. Mean antibody prevalences were: 60.6 ± 0.06% for Aujeszky's disease virus (ADV), 56.6 ± 0.09% for porcine parvovirus (PPV), 51.8 ± 0.06% for porcine circovirus type 2 (PCV2), 29.7 ± 0.09% for *Brucella* spp. and 36.3 ± 0.1% for *Toxoplasma gondii*. We did not detect antibodies against porcine

reproductive and respiratory syndrome virus (PRRSV). ADV seroprevalence was associated with PPV and PCV2 seroprevalence in Spanish wild boar females. Ovulation rate in the studied wild boar females was 4.41 ± 0.16 (n = 120), mean litter size was 3.91 ± 0.16 (n=82) and the partial resorption index 0.92 ± 0.17 (n = 66). Ovulation rate and litter size were statistically associated with age. *T. gondii* seroprevalence was negatively related to ovulation rate and partial resorption index. Wild boars from managed fenced estates had antibodies against more pathogens than wild boars from open estates. Potential relations between management of wild boar populations and exposure of individuals to different reproductive pathogens are discussed.

Keywords: Infectious diseases; Reproduction; Seroprevalence; Wild boar; Spain

Introduction

Populations of the European wild boar (*Sus scrofa*) have largely increased in Spain during the past 30 years (Saez-Royuela and Tellería, 1986; Gortázar et al., 2000). In terms of hunting harvest, the Iberian red deer (*Cervus elaphus hispanicus)* and European wild boar are at present the most important big game species in Spain. Due to its economic relevance, many of the Spanish wild boar populations are subject to management in order to increase hunting harvest. Practices, such as high-wire fencing, artificial feeding and restocking, are becoming more common, while sanitary measures are not implemented to match this development. As a result, elevated boar densities are found that have already been shown to imply consequences for the control of infectious diseases (Gortazar et al., 2005, 2002; Vicente et al., 2004, 2005, **Capítulo 1.1**).

The European wild boar is a polygynous species with an autumnal breeding season influenced by environmental conditions. The Spanish wild boar breeding season occurs between September and December, with a main peak in October (Rosell et al.,

2001). Farrowing takes place after 120 days in January–March, although some boars farrow in August–September.

The domestic pig and wild boar share common pathogens (Lipowski, 2003; Mason and Fleming, 1999). The European wild boar could constitute a disease reservoir for the domestic pig (Cvetnic et al., 2003). Some of these pathogens produce reproductive failure in pregnant sows, reducing litter size or killing the whole litter. Return to estrus, resorption, mummification, abortion and fetal death are described in *S. scrofa* as the main features of diseases that affect reproduction. However, the knowledge about the epidemiology of reproductive diseases in wild boars is still limited.

The most relevant viral diseases that produce reproductive failure in domestic pigs are porcine reproductive and respiratory syndrome (PRRS) and porcine parvovirosis (PPV) (Mengeling et al., 2000). Other pathogens affect the reproductive biology of suids, including viral, bacterial and parasitic agents. Aujeszky's disease virus (ADV) or pseudorabies virus infects the domestic pig, wild boar and feral pig. Although Aujeszky's disease clinical signs are primarily nervous and respiratory, the virus has a tropism for the reproductive tract and causes reproductive losses in pregnant domestic sows (Kluge et al., 1999). PPV is an ubiquitous and resistant virus with a worldwide distribution. While in immune adult animals reproductive effects are generally not detected, the tropism of the virus for the reproductive tract may lead to mummified fetuses and resorptions in naïve females, especially in their first pregnancy (Mengeling et al., 2000). Porcine circovirus type 2 (PCV2) is considered the cause for postweaning multisystemic wasting syndrome (PMWS) (Segalés et al., 2004). A reproductive effect of PCV2 has been described in domestic sows (West et al., 1999), with abortion, infected stillborn and non-viable neonate piglets. Porcine reproductive and

respiratory syndrome virus (PRRSV) is also widely distributed among domestic pigs and has been associated with reproductive failure in domestic gilts. PRRSV infection can cause resorption, late-term abortions, stillborn and weak piglets in pregnant sows (Benfield et al., 1999). *Brucella* is a worldwide distributed bacterial genus which produces reproductive failure in mammals (Godfroid and Käsbohrer, 2002). Porcine brucellosis in Europe is mainly caused by *Brucella suis* biovar 2 while the disease is caused by biovars 1 and 3 in Asia and America (Becker et al., 1978; Van der Giessen and Priadi, 1988; Cornell et al., 1989; Lord et al., 1997). Brucellosis due to *B. suis* biovar 2 has been considered a re-emerging disease in domestic pigs in Europe, caused by spillover from the wild boar (Godfroid and Käsbohrer, 2002). *B. suis* biovar 2 has been isolated from European wild boars (Cvetnic et al., 2003; Garin-Bastuji et al., 2000) in which no clinical disease was observed. Finally, *Toxoplasma gondii* is a pathogenic protozoan that infects a wide range of hosts, causing reproductive failure in females which contract the parasite for the first time during pregnancy. Its implication in abortion in domestic sows, however, is uncommon (Lindsay et al., 1999). To our knowledge, few serological studies exist on the prevalence of antibodies against *T. gondii* in the wild boar (Diderrich et al., 1996; Edelhofer et al., 1996; Gauss et al., 2005).

Our goals were to describe risk factors that explain the presence of antibodies against these six significant pathogens among wild boars in Spain and to determine, by means of a correlational study, the potential influence that these diseases may have on reproductive parameters in wild boar females.

Material and methods

Sampling sites and field necropsies

Data were collected from hunter-harvested wild boars in 54 Spanish hunting estates during the hunting seasons from 2000 to 2003. The sampling effort was biased towards the main hunting season (October–February).

Every animal was morphometrically characterized, weighed and necropsied in detail. We determined the age of the wild boars on the basis of tooth eruption patterns (Saenz de Buruaga et al., 1991). Wild boars between 7 and 12 months were classified as juveniles, those between 12 and 24 months as sub-adults, and those over 2 years as adults. Blood was collected from the heart into sterile tubes, left to coagulate and maintained at 4 ºC until arrival at the laboratory. Serum was separated by centrifugation of the blood samples and stored frozen at -20 ºC. Reproductive tracts were collected from all females and inspected in the laboratory. Ovarian activity was recorded and the fetuses found were sexed, measured and weighed. Ovulation rate was defined as the number of *corpora lutea* found in both ovaries and litter size was defined as the number of fetuses found in the uterus (Abaigar, 1992). A partial resorption index was established in pregnant females calculating the difference between number of viable fetuses and number of *corpora lutea* observed in both ovaries. We parted from the fact that wild boar females become pregnant for their first time at the age of 8–10 months (Rosell et al., 2001). Thus, sub-adult females estimated to be less than 18 months old, based on tooth eruption patterns and morphometry (total length), were classified as primiparous, whereas females estimated to be elder subadults and adults where considered multiparous. Sub-adult females' total length presented a bimodal distribution (114 and 123 cm as modes, respectively). We decided not to include pregnant juvenile females due to the low number found (n = 3).

Hunting estates were classified as open or fenced according to data obtained through a personal interview with the gamekeepers. No management at all is

applied to wild boar in open areas, while in areas classified as fenced, feeding is a common practice.

Serological analyses

Serum samples were tested to detect ADV, PPV, PCV2, PRRSV, *Brucella* spp. and *T. gondii* antibodies. Data regarding serological analyses for antibodies against ADV, PCV2 and *T. gondii* were previously reported by our group (Vicente et al., 2004, 2005, **Capítulo 1.1**; Gauss et al., 2005). Serological data presented here concern only wild boar females. Sera (n=284) were analysed for ADV antibodies using a commercial blocking ELISA based on the detection of specific anti-gE antibodies (Chekit Aujeszky-ELISA, Bommeli Diagnostics, Switzerland) following the manufacturer's recommendations. This ELISA technique has been previously used in wild boar (Vicente et al., 2005, **Capítulo 1.1**) and it has a great sensitivity and specificity (95.2–98.9% and 97.8–99.5%, respectively). To detect PPV antibodies, 129 sera were tested by means of a commercial blocking ELISA test (ELISA PPV compac-INGENASA, Spain). This blocking ELISA detects antibodies against the VP2 protein. This test reaches values of 100% sensitivity and 98.8% specificity. By means of an immunoperoxidase monolayer assay (IPMA) test, 272 sera were tested against PCV2 antibodies as previously described in wild boar (Vicente et al., 2004). The sensitivity and specificity of the IPMA test are comparable to those of the ORF-2 protein-based ELISA test (Blanchard et al., 2003), with values of 98.2% and 94.5%, respectively. Also, 123 sera were analysed for the presence of PRRSV antibodies (HerdCheck* PRRS Virus Antibody Test Kit 2XR, IDEXX, USA). This test has a high sensitivity and specificity (97.5% and 99.5%, respectively) and has been previously used in European wild boar (Zupanzic et al., 2002). We also tested 118 sera against *Brucella* spp. antibodies by means of the Rose Bengal test (RBT), as described in the OIE manual of

standards (http://www.OIE.int), and 91 sera by means of a modified agglutination test (MAT) (Dubey and Desmonts, 1987) for *T. gondii* antibodies. To detect *T. gondii* antibodies sera were diluted to 1:25, 1:50 and 1:500, and incubated with mercaptoethanol at 37 ºC during 8 h. Sera reacting at 1:25 or above were considered positive. MAT is the most sensitive and specific test for the serodiagnosis of toxoplasmosis in swine (Dubey et al., 1995). Seventy-seven sera were analysed for all of the pathogens studied. We defined an index called mean index of pathogen seroprevalence (MIPS) that states the number of different pathogens against which any of these 77 individuals had antibodies. This index ranged from 0 (no antibodies against any of the tested pathogens) to 6 (antibodies against all of the studied pathogens).

In our study, all analyses are based on the seroprevalence of antibodies against the different pathogens, which implies that we are most probably slightly underestimating the real number of infected animals. This is especially true for infections with *Brucella* spp. The temporal absence of antibodies in adult infected females implies that we underestimate the true number of infected animals.

Statistical analyses

Confidence intervals (C.I.) for standard errors of the seroprevalence of antibodies against the different pathogens were estimated using the expression $S.E._{95\%C.I.} = 1.96[p(1-p)]/n^{1/2}$ (Martin et al., 1987). The association between antibody seroprevalence against different diseases and differences between sub-adults and adults regarding the seroprevalence of antibodies (except for antibodies against PRRSV) were tested by means of a Chi-square test to compare expected with observed values (Stone and Pence, 1978). Differences between sub-adults and adults regarding pathogen seroprevalence (except PRRSv) were tested by means of a Chi-square test. Sub-adults and adults of the

same estate type were analysed separately. Females from both estate types and both age classes were grouped for the analysis. We designed general mixed linear models (GLIMMIX, Wilson and Grenfell, 1997) to analyse the influence of the considered variables on the female reproductive parameters (ovulation rate, litter size and partial resorption index). Prior to the analysis, we standardized the effect of explanatory variables on the different reproductive parameters, excluding the seroprevalence of antibodies against pathogens. First, we standardized the effect of explanatory parameters except infection on the different reproductive parameters tested separately as dependent variables. Explanatory variables included age (sub-adult or adult, as continuous variable), body condition (kidney fat index (KFI) as continuous variable, %), estate management (categorical as open or fenced) and previous reproductive life (categorical as primiparous or multiparous). Since we carried out the analysis at the individual level, in order to control for the effect of sampling month and area of origin (group of populations from a particular geographical unit: Sierra Morena, Toledo Mountains, Guadiana), we included both month and area as random effects. This enabled us to control for population effects in order to achieve a true individual-based analysis. Subsequently, we tested the serological status of the individuals against each of the tested pathogens and the MIPS separately by means of a GLIMMIX. We designed similar models to those described but including the exposition to the pathogen (seroprevalence status as categorical variables, 0=seronegative; 1=seropositive) and their interactions as explanatory variables. Similarly, area and month (as categorical variables) were considered as random. We considered a Poisson error and a logarithmic link function (Wilson and Grenfell, 1997) since dependent variables always yielded $p<0.05$ at Shapiro–Wilk's W-test and at the Kolmogorov–Smirnov test for all age classes. A backward stepwise procedure was followed to exclude non-significant

variables or interactions. We also tested for the effect of the intensity of management (open versus fenced) on seroprevalence of antibodies at estate level (Chi-square test) and for differences in MIPS between age classes and degree of management (Mann–Whitney U-test). The statistical significance of the p-value was set at 0.05.

Results

Reproductive parameters and antibody prevalences against the tested pathogens according to age class and estate type, as well as the significance of their variations, are shown in Table 1. No PRRSV antibodies were detected in any of the 123 sera analysed.

Risk factors for pathogen exposure

The seroprevalences of antibodies against ADV and PCV2 differed significantly between both age class and management type. *T. gondii* antibody seroprevalence was significantly higher in adult (55.2 ± 9%) than in sub-adult (27.8 ± 7%) females from fenced estates. Grouping both estate types, adult females had significantly higher seroprevalences of antibodies against PPV than sub-adults. We found statistical differences between open and fenced estates regarding the MIPS for each age class and for both together. The highest values for this index were found in adult females from fenced estates (Table 1; Fig. 1).

The presence of antibodies against ADV was statistically associated with seropositivity against PPV and PCV2, as shown in Table 2.

Risk factors of reproductive failure

Statistically significant differences in ovulation rate and litter size between adult and sub-adult females were observed independently of the seroprevalence of antibodies against any pathogen. Higher ovulation rate and litter size values were observed in adult

females. No other explanatory variables were found to significantly affect ovulation rate, litter size and partial resorption in our model.

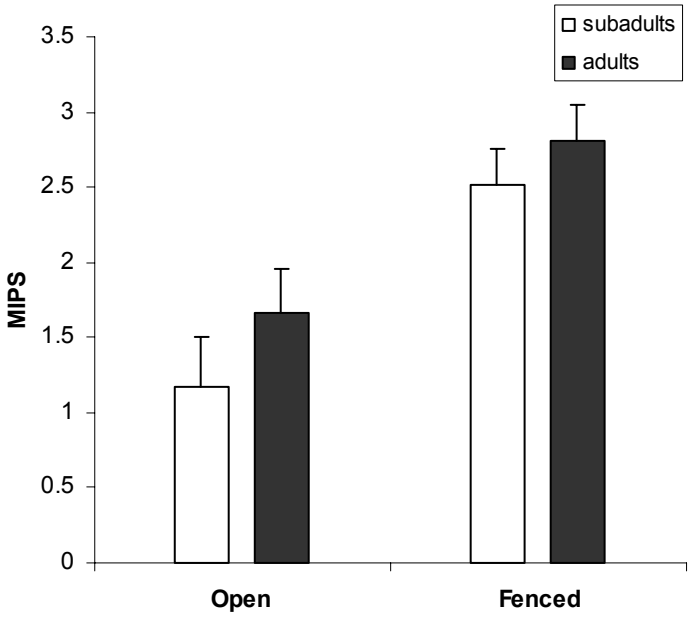

Figure 1: Differences found in MIPS (± SEM) through age-by-management groups. Statistical differences were found between open and fenced estates ($p<0.05$) as shown in Table 1.

When in a second model the seroprevalence of antibodies against the studied pathogens was included, a statistically significant relationship was found between *T. gondii* antibody seroprevalence and the partial resorption index and ovulation rate. *T. gondii* seronegative gravid females had more resorptions and lower ovulation rate values than seropositive ones ($p=0.05$, $n=24$ and $p<0.05$, $n=46$, respectively). Also, we found that the interaction between the MIPS and age significantly influenced the ovulation rate ($p<0.05$). This effect was more pronounced in adult than in sub-adult females (linear regression: $B=0.50$, $r=0.288$; $B=0.09$, $r=0.06$, respectively). No statistically significant relationships were evidenced regarding the prevalence of antibodies against ADV, PPV, PCV2 and *Brucella* spp. and any of the reproductive parameters. The related data are shown in Table 3.

Table 1. Mean reproductive parameters (mean ± S.E) and seroprevalence (mean ± S.E , %) for different pathogens and MIPS through age-by-management groups. We display the p values of statistical tests.

	OPEN ESTATES			FENCED ESTATES			TOTAL		
	Sub-adults	**Adults**	**Total**	**Sub-adults**	**Adults**	**Total**	**Sub-adults**	**Adults**	**Total**
Ovulation rate	4.24 ± 0.43 (n=17)	4.85 ± 0.72 (n=13)	4.5 ± 0.39 (n=30)	3.95 ± 0.22[a] (n=44)	4.78 ± 0.25[a] (n=46)	4.38 ± 0.17 (n=90)	4.03 ± 0.19[d] (n=61)	4.8 ± 0.25[d] (n=59)	4.41 ± 0.16 (n=120)
Litter size	3.57 ± 0.53 (n=7)	4.63 ± 0.5 (n=8)	4.13 ± 0.38 (n=15)	3.43 ± 0.21[a] (n=28)	4.18 ± 0.26[a] (n=39)	3.87 ± 0.18 (n=67)	3.46 ± 0.19[d] (n=35)	4.26 ± 0.27[d] (n=47)	3.91 ± 0.16 (n=82)
Partial resorption index	1.2 ± 0.73 (n=5	0.5 ± 0.34 (n=6)	0.82 ± 0.38 (n=11)	0.82 ± 0.23 (n=28)	1.07 ± 0.3 (n=27)	0.95 ± 0.19 (n=55)	0.88 ± 0.22 (n=33)	0.97 ± 0.26 (n=33)	0.92 ± 0.17 (n=66)
ADV	35.7 ± 7 %[b] (n=42)	54.2 ± 10 %[b] (n=24)	42.4 ± 6 %[c] (n=66)	55.9 ± 5 %[a,b] (n=102)	75 ± 4 %[a,b] (n=116)	66.1 ± 3 %[c] (n=218)	50 ± 4 %[d] (n=144)	71.4 ± 4 %[d] (n=140)	60.6 ± 3 % (n=284)
PPV	45 ± 11 % (n=20)	60 ± 13 % (n=15)	51.4 ± 9 % (n=35)	50 ± 7 % (n=50)	68.2 ± 7 % (n=44)	58.5 ± 5 % (n=94)	48.6 ± 6 %[d] (n=70)	66.1 ± 6 %[d] (n=59)	56.6 ± 4 % (n=129)
PCV 2	25.7 ± 8 %[b] (n=35)	31.8 ± 10 %[b] (n=22)	28.1 ± 6 %[c] (n=57)	59.6 ± 5 %[b] (n=99)	56.9 ± 5 %[b] (n=116)	58.1 ± 3 %[c] (n=215)	50.7 ± 4 % (n=134)	52.9 ± 4 % (n=138)	51.8 ± 3 % (n=272)
PRRSV	0 ± 0 % (n=20)	0 ± 0 % (n=15)	0 ± 0 % (n=35)	0 ± 0 % (n=47)	0 ± 0 % (n=41)	0 ± 0 % (n=88)	0 ± 0 % (n=67)	0 ± 0 % (n=56)	0 ± 0 % (n=123)
***Brucella* spp.**	10 ± 7 % (n=20)	28.6 ± 13 % (n=14)	17.6 ± 7 % (n=34)	31.9 ± 7 % (n=47)	37.8 ± 8 % (n=37)	34.5 ± 5 % (n=84)	25.4 ± 5 % (n=67)	35.3 ± 7 % (n=51)	29.7 ± 4 % (n=118)
T. gondii	23.1 ± 12 % (n=13)	30.8 ± 13 % (n=13)	26.9 ± 9 % (n=26)	27.8 ± 7 %[a] (n=36)	55.2 ± 9 %[a] (n=29)	40 ± 6 % (n=65)	26.5 ± 6 % (n=49)	47.6 ± 8 % (n=42)	36.3 ± 5 % (n=91)
MIPS	1.17 ± 0.34[b] (n=12)	1.67 ± 0.28[b] (n=12)	1.42 ± 0.22[c] (n=24)	2.52 ± 0.24[b] (n=31)	2.81 ± 0.24[b] (n=22)	2.64 ± 0.17[c] (n=53)	2.14 ± 0.22 (n=43)	2.41 ± 0.21 (n=34)	2.26 ± 0.15 (n=77)

a) Significant differences between sub-adults and adults from the same estate type ($p<0.05$).
b) Significant differences between similar aged animals from open and fenced estates ($p<0.05$).
c) Significant differences between estate type for adults and sub-adults together ($p<0.05$).
d) Significant differences between age classes considering both estate types together ($p<0.05$).

Table 2: Association of antibody seroprevalence against different pathogens. We display the positives and the total number of analysed animals for both pathogens. The statistical relationship between seroprevalence of antibodies against the pathogens in all animals considered is shown.

	ADV	PPV	PCV2	*Brucella*	*T. gondii*
ADV		52 (120), χ^2=9.02*	96 (252), χ^2=91.7***	22 (112), χ^2=0.5	19 (83), χ^2=1.47
PPV			38 (114), χ^2=1.35	18 (110), χ^2=0.0	23 (82), χ^2=0.46
PCV2				22 (106), χ^2=0.5	16 (79), χ^2=3.2
Brucella					10 (76), χ^2=0.45
T. gondii					

* **p<0.05**
*** **p<0.001**

Discussion

Many pathogens are able to infect both, wild and domestic animals, and may be transmitted between them, which has been demonstrated specifically for the wild boar and the domestic pig (Müller et al., 2001). Some of the diseases suffered by swine have effects on the reproductive function, leading to important economic losses in pig production (Straw et al., 1999). In this study, we tested the seroprevalence of antibodies against some of the most significant pathogens known to be responsible for reproductive failure in domestic pigs and that could potentially lead to similar effects in the wild boar.

Table 3: GLIMMIX models for ovulation rate, litter size and partial resorption index in wild boar females. Pathogen antibody prevalence status was not included in the first models. Secondly, each antibody prevalence against each pathogen was considered separately. We used Poisson error and logarithmic link. The p-values of the statistical tests are shown.

Dependent variables	Explanatory variables (final models)	Num Df/Den Df	F	*p*	Param. Est. ± E.S.[a]
Pathogens` status not considered					
Ovulation rate (*n*=107)	Age[b]	1/105	6.15	0.01	0.17 ± 0.07
Litter size (*n*=75)	Age[b]	1/73	6.66	0.01	0.22 ± 0.09
Partial resorption (*n*=63)	No significant explanatory variables				
ADV					
Ovulation rate (*n*=92)	Age[b]	1/90	6.83	0.01	0.20 ± 0.07
Litter size (*n*=65)	Age[b]	1/63	6.55	0.01	0.24 ± 0.09
Partial resorption (*n*=53)	No significant explanatory variables				
PCV2					
Ovulation rate (*n*=90)	Age[b]	1/88	6.75	0.01	0.19 ± 0.07
Litter size (*n*=60)	Age[b]	1/58	6.90	0.01	0.26 ± 0.1
Partial resorption (*n*=49)	No significant explanatory variables				
PPV					
Ovulation rate (*n*=68)	PPV[c]	1/63.2	3.65	0.06[(c)]	-0.16 ± 0.09
	Age[b]	1/63.8	6.82	0.01	0.23 ± 0.09
Litter size (*n*=48)	No significant explanatory variables				
Partial resorption (*n*=37)	No significant explanatory variables				
Brucella					
Ovulation rate (*n*=61)	Age[b]	1/57.5	7.19	0.01	0.25 ± 0.09
Litter size (*n*=45)	Age[b]	1/41.8	4.67	0.03	0.13 ± 0.13
Partial resorption (*n*=34)	No significant explanatory variables				
Toxoplasma gondii					
Ovulation rate (*n*=46)	TOX[e]	1/41.6	4.55	0.03	-0.24 ± 0.11
	Age[b]	1/40.6	5.52	0.02	0.27 ± 0.1
Litter size (*n*=30)	No significant explanatory variables				
Partial resorption (*n*=24)	TOX[e]	1/20.1	4.31	0.05	1.38 ± 0.67
Mean index of pathogen seroprevalence (MIPS)					
Ovulation rate (n=42)	Estate type	1/38.9	4.5	0.04	0.41 ± 0.15
	MIPS*Age	1/36	5.08	0.03	0.03 ± 0.01
Litter size (*n*=75)	No significant explanatory variables				
Partial resorption (*n*=23)	No significant explanatory variables				

[a] Parameter estimate ± standard error.
[b] Age class (sub-adults and adults).
[c] PPV serological status.
[d] Marginally significant.
[e] *T. gondii* serological status.

Although the wild boar and the domestic pig could be considered as the same species, the disease epidemiology varies between wild and domestic animals due to the different associated risk factors (Millán et al., 2004). Most of the tests used in this work have been previously used in wild boar although they were primarily developed to test domestic pig sera. The number of sera analysed for antibodies against each pathogen in this work varies due to problems during the sampling period. Nonetheless, we used GLIMMIX to avoid mixed influences of factors on results and confounding effects. Thus, sampling bias exists, that could hide some deeper effect of the diseases on the population dynamics of the wild boar. Also, a prolongation of this study in time could provide more information on variability in the exposure to pathogens and consequences on the productivity over the long term. The results for reproductive parameters found in our study are comparable with other European studies (Mauget, 1972; Rosell et al., 2001; Abaigar, 1992). In the females analysed, ovulation rate was slightly lower when compared to most of the published studies, while litter size in open estates was more similar to other European populations (Mauget, 1972; Aumaitre et al., 1982). Litter size in fenced estates was very low and only comparable to those recorded in Doñana National Park (in the southern limit of Spain) during a drought period (Fernández-Llario and Carranza, 2000).

Antibodies against ADV were extensely prevalent in the tested wild boar females. No statistical relationship could be established between ADV seroprevalence and reproductive parameters. ADV can produce resorption, mummification and abortion in pregnant sows (Kluge et al., 1999). Previous studies have shown that reproductive effects are not common in ADV infections in domestic pigs, but a primary outbreak in an immunologically unprotected herd caused abortion in almost 20% of the pregnant females (Kluge et al., 1999).

Antibodies against PPV were widely distributed among the wild boar females in our study. Reproductive failure due to PPV occurs if infection is contracted during the first half of gestation, while infection after 70 days of gestation can lead to weak, infected piglets (Mengeling et al., 2000). In our study, seroprevalence of antibodies against this virus was not related to values for any reproductive parameter, nor did it differ between open and fenced estates. This may be due to a high transmission rate of PPV between the wild boars, even in populations of low density. However, more information would be necessary to elucidate the importance of PPV in Spanish wild boar populations.

PCV2 can infect the reproductive tract of females causing reproductive failure (West et al., 1999). Almost all descriptions of reproductive failure due to PCV2 in domestic pigs have been reported in Canada, with very few cases in the rest of the pig producing countries (Segalés et al., 2004). Little information is available regarding PCV2 infections in the wild boar (Schulze et al., 2003; Vicente et al., 2004). No antibodies against PRRSV were detected in our sera, which indicates that PRRSV has little or no importance in Spanish wild boar populations. This finding is similar to reports from Croatia (Zupanzic et al., 2002) and Germany (Lutz and Wurm, 1996), but it differs from other European studies (Oslage et al., 1994; Albina et al., 2000). It is also in contrast to the high prevalence of PRRSV reported for Spanish herds of domestic pigs (Gutiérrez-Martín et al., 2000).

We used the RBT to determine the presence of antibodies against *Brucella* spp. because this test had been previously employed with sera from wild boars (Ebani et al., 2003). Although RBT is not recommended for the diagnosis of exposure to *Brucella* spp. in individual wild boars, it is considered adequate to determine the status of a population. No statistically significant relationship has been found in this study

between the seroprevalence of antibodies against *Brucella* spp. and any of the reproductive parameters. *Brucella* spp. is known to produce abortion at all stages of gestation, generally of the whole litter. Since we were only able to study effects on partial resorption and litter size, other adverse effects of *Brucella* spp. on wild boar reproduction, especially the latter, may have gone undetected. Nevertheless, the high seroprevalence found (29.7%) suggests, in contrast to previous data (Vicente et al., 2002), that *Brucella* should be further studied in Spanish wild boar.

If infection with *T. gondii* in the domestic pig occurs during gestation, this can lead to abortion, although this is not considered common (Lindsay et al., 1999). In our study, females seropositive against *T. gondii* had lower values of the partial resorption index than seronegative females. The fact that *T. gondii* produces abortion only in a primary infection during pregnancy could explain our results.

Concerning ovulation rate, the observed positive effect of MIPS, particularly in adult females, could be due to an increased risk of exposition to infectious agents across age rather than a real effect of MIPS. In addition, reproductive diseases are more likely to affect embrionary or fetal development than ovarian activity. Seroprevalence of antibodies was higher in fenced estates for each of the pathogens tested. Fencing is generally associated with feeding of wild animals and usually supposes an increment in their densities (Vicente et al., 2004). It generally also causes aggregation, thus increasing contact among wild boars (the authors, unpublished observations). Hence, current hunting management systems may be contributing to the maintenance of the circulation of certain pathogens in wild boar populations. We also observed differences in the MIPS between open and fenced estates. This finding suggests that animals from fenced estates are more prone to suffer from multiple infections than those from open estates. This is especially relevant in wild boar disease control and management

programmes. Mixed infections with different pathogens are common in domestic pig production (Pejsak and Markowska-Daniel, 2003). In some of these cases, spread of the pathogens is favoured by high densities of animals (Mangen et al., 2002). Current hunting management systems represent increased disease risks not only for the wild boar, but also for other wildlife, domestic animals and man (Gortázar et al., 2005). Hence, veterinary authorities and wildlife managers should consider limiting those management measures that lead to wild boar overabundance.

The studied pathogens may have only a limited role in the reproductive performance of Spanish wild boar females. However, the wild boar seems to participate in the maintenance of ADV, PPV, PCV2, *Brucella* spp. and *T. gondii* in Spanish mainland. The importance of these pathogens, especially ADV, for the pig production in Spain makes the wild boar a species to take into account in pig disease control programmes. Nonetheless, more work is needed to determine the epidemiology of these diseases in the European wild boar in Spain and their role in Animal Health.

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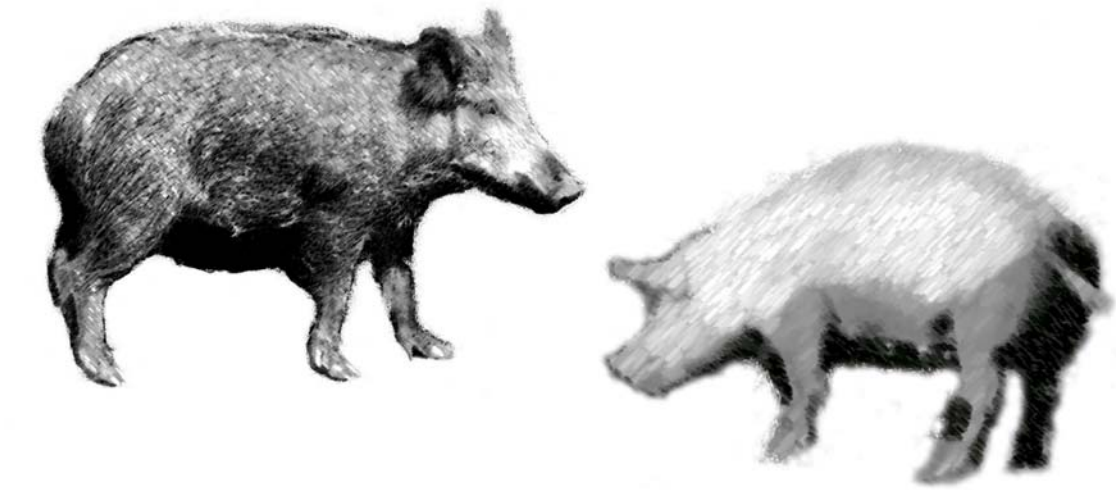
Capítulo 4

Interacciones epidemiológicas sobre la enfermedad de Aujeszky entre el porcino doméstico y el jabalí en Castilla-La Mancha, España



Francisco Ruiz-Fons, Dolo Vidal, Joaquín Vicente, Pelayo Acevedo, Isabel G. Fernández-de-Mera, Vidal Montoro, Christian Gortázar

Epidemiological interactions on Aujeszky's disease between domestic pig and European wild boar in Castilla-La Mancha, Spain

Enviado a: ***Preventive Veterinary Medicine***

Resumen

La campaña de control y erradicación de la enfermedad de Aujeszky empezó en España en el año 1995 y, como resultado, las seroprevalencias del virus de la enfermedad de Aujeszky (ADV) han disminuido notablemente en las granjas de cerdos españolas, aunque la erradicación todavía no es un hecho. Como las seroprevalencias del ADV en las poblaciones españolas de jabalíes pueden suponer un impedimento para los esquemas de erradicación del ADV en el cerdo doméstico, realizamos un análisis de factores de riesgo e investigamos las asociaciones entre los patrones de seroprevalencia del ADV a nivel municipal en el jabalí y en el cerdo doméstico, respectivamente, en el centro-sur de España. Hubo un patrón claro de incremento con la edad estadísticamente significativo en el jabalí, y las hembras presentaron un mayor riesgo estadístico de resultar positivas que los machos. El análisis de factores señaló que el incremento de la cobertura arbórea se relacionó estadística y positivamente con el riesgo individual de resultar positivo al ADV. En relación al modelo realizado para el cerdo, se observaron mayores seroprevalencias del ADV a lo largo del incremento en el número de cerdos por granja. Las granjas cerradas de cerdos mostraron estadísticamente mayores seroprevalencias que en las abiertas. Tanto en el jabalí como en el cerdo doméstico se observó autocorrelación espacial a nivel de término municipal, con mejores distancias de ajuste de 25.6 y 9.5 km., respectivamente. Se concluye que, al menos a la escala de estudio, no hubo ninguna evidencia de interacción entre la epidemiología de la enfermedad de Aujeszky en cerdo doméstico y jabalí. Estos resultados son relevantes tanto para las autoridades veterinarias como para las de salud pública, aunque un mayor esfuerzo es necesario para determinar la naturaleza de brotes concretos de la enfermedad de Aujeszky, lo que probablemente requiera una aproximación molecular.

Abstract

An Aujeszky's disease (AD) control and eradication campaign in domestic pigs started in Spain in 1995, and as a result ADV seroprevalences have notably diminished in Spanish domestic pig herds, but eradication has still not been achieved. Since ADV seroprevalences in Spanish wild boar populations can impede schemes to eradicate ADV in domestic pig, we conducted analysis of risk factors and investigated associations between the patterns of ADV seroprevalence at municipal level in the wild boar and the domestic pig, respectively, in south-central Spain. There was a clear statistically significant age increasing pattern in wild boar, and females presented statistically higher risk of testing positive than males. The risk factor analysis yielded that increasing tree cover statistically and positively related to the individual risk of testing positive to ADV. Concerning the domestic pig model, higher ADV seroprevalences were observed along the increasing number of pigs per farm. Indoor pig farms statistically showed higher seroprevalences than open-air farms. In both in wild boar and domestic pig there was spatial autocorrelation at municipality level, with best fits at lag distances of 25.6 km and 9.5 km, respectively. It is concluded that, at least at the study scale, there was not any evidence of interaction between the epidemiology of AD in the domestic pig and the wild boar. These findings are of concern for both veterinary and public health authorities, but more effort is needed in other to elucidate the nature of particular ADV outbreaks, which probably will require a molecular approach.

Keywords: Domestic pig; Pseudorabies; Risk assessment; Seroprevalence; Wildlife.

Introduction

Aujeszky´s disease (AD) or pseudorabies is caused by porcine *Herpesvirus* type I. AD virus (ADV) infects wild and domestic swine as natural hosts but also a wide range of domestic and wild mammals (Kluge et al., 1999). The disease is worldwide spread and causes big economic losses due to its direct effect on domestic pigs and indirect impact on the international trade of porcine products (Moynagh, 1997). Many European countries have become ADV free in their domestic pig herds (Moynagh, 1997; Müller et al., 2001) as a consequence of successful control and eradication programs (e.g. The Netherlands, Denmark or Germany). In Spain a vaccination campaign with gE-deleted attenuated vaccines was implemented in 1995 (http://www.mapya.es). Reproductive animals from seropositive herds are of obligated vaccination 3 times a year and breeding animals are obligatorily vaccinated two times at 10-12 weeks of life and 3 to 4 weeks later with a second revaccination for those older than 6 months.

Epidemiological studies on ADV infection in domestic pigs have shown that management systems, topographical features, the distance between pig farms, pig density and pig farm density among others are important risk factors (Marsh et al., 1991; Austin and Weigel, 1992; Leontides et al., 1994; Tamba et al., 2002). Epidemiological studies on ADV seroprevalence have also been carried out in wild boar (Müller et al., 1998; Lutz et al., 2003), although the role of domestic pigs has not been assessed before. Intensive game management could incur an increased risk for ADV spread within populations (Vicente et al., 2005, **Capítulo 1.1**).

The European wild boar is one of the most abundant ungulate species on the Spanish mainland. Wild boar populations have largely increased in Spain during the last 30 years (Saez-Royuela and Tellería, 1986; Gortázar et al., 2000; Acevedo et al., 2006). In order to increase hunting harvests, many wild boar populations in South Central

Spain have essentially become captive or semi-domestic, and artificial feeding and watering is usually provided during all or part of the year, resembling those managements of open air domestic pigs, but without the sanitary care. Also, wild boar translocations are frequent in order to increase hunting harvest and to avoid inbreeding, which involves sanitary risks (Fernández de Mera et al., 2003). More than 40% of the Spanish wild boars presented antibodies against ADV (Vicente et al., 2005, **Capítulo 1.1**), with higher values in South central Spain. Also, an ADV outbreak was described in this area (Gortázar et al., 2002). No ADV antibodies were detected in the northern areas of Spain (Vicente et al., 2005, **Capítulo 1.1**), although low seroprevalences (8.5%) have been detected in north-eastern Spain (M. C. Arnal, personal communication). The wild boar is capable to act as an ADV reservoir (Lipowski, 2003; Lutz et al., 2003). The experimental infection of domestic pigs with ADV strains of wild boar origin (Müller et al., 2001) and the excretion of viruses to the environment by wild boars (Müller et al., 1998) raise the possibility of between-suids ADV transmission. A recent AD outbreak in an ADV-free open-air domestic pig farm from the French department of Loiret was suggested to be caused by contacts with wild boars (Hars and Rossi, 2005). Open-air production of Iberian pigs is traditional in south-western Spain, where high densities of wild boar are concurrent (Höfle et al., 2004). This particular situation could imply risk of ADV transmission at the wild boar/Iberian domestic pig interface. This especial feature stresses that evidencing any shared epidemiology of ADV between the wild boar and the domestic pigs in Spain may reveal crucial in order to eradicate AD. Nevertheless, the role of the wild boar in AD outbreaks in domestic pigs in Germany was discarded based on molecular evidence (Müller et al., 1997). Moreover, Germany was declared as ADV-free in their domestic pigs despite the circulation of ADV among wild boar populations (Lutz et al., 2003).

By means of exploratory analyses, our main goal was to investigate the associations between the patterns of ADV seroprevalence at municipal level in the wild boar and the domestic pig in a region of south-central Spain.

Material and methods

Study area

The region of Castilla–La Mancha (CLM) is located in the south of the Central Spanish Plateau. The climate is Mediterranean with a continental influence and annual rainfall is extremely variable (ranging from 300 to 700 mm). The wet season typically starts in September-October and contributes most of the annual rainfall. Across this region, big game hunting estates are allocated on woodlands. Grossly, the habitat is Mediterranean and characterized by evergreen oak (*Quercus ilex*) forests and scrublands (dominated by *Cystus* spp., *Pistacia* spp., *Rosmarinus* spp., *Erica* spp. and *Phyllirea* spp.) with scattered pastures and small areas of crops. The northeasternmost areas are characterized by pine (*Pinus* spp.) and holm oak (*Quercus faginea*) forests, whereas in the south-west of the study region mainly predominate evergreen oak. The south east of the study region is characterized by high elevations (up to 1,600 m.a.s.l.) with mixed evergreen oak and pine forests. Large agricultural lands are present between woodlands and represent the most common habitat use in the centre of the study region.

Wild boar data

From 1999 to 2005 wild boars (n=1,714) were sampled in 74 public and private hunting estates from CLM. Hunting estates were grouped into municipalities. Samples were biased towards the main hunting season (from October to February). Wild boars were mainly taken from Montes de Toledo (MT, n=693), Sierra Morena (SM, n=539) and Guadiana valley (GU, n=324). These areas, together with the Toledo province (TO,

n=74), present a higher management intensity and wild boar densities as compared to Guadalajara (GJ, n=17), Cuenca (CU, n=34) or Albacete (AB, n=33).

In the field, a necropsy was performed, including detailed determination of morphometry, weight, and sex. Based on tooth eruption patterns, animals younger than 6 month old were classified as piglets, boars between 7 and 12 months were classified as either juveniles, sub-adults of between 12 and 24 months, or adults of over 2 years old (Saenz de Buruaga et al., 1991).

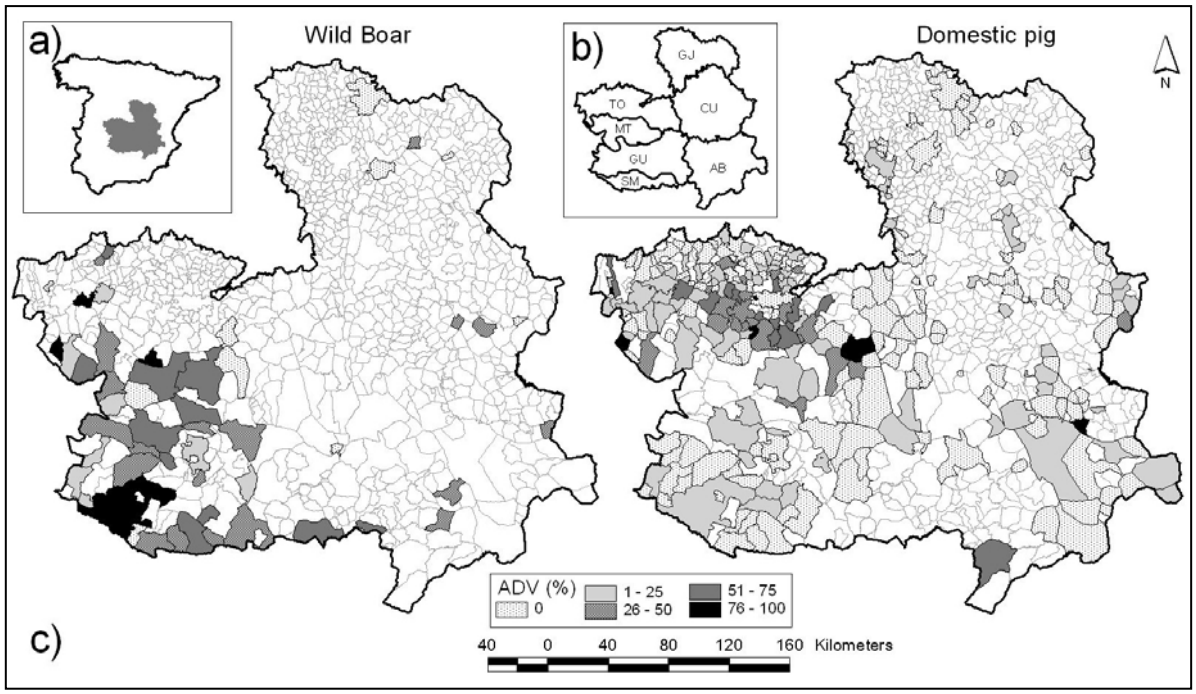


Figure 1. Location of the study area, Castilla-La Mancha (a), geographical areas considered (b), and ADV seroprevalences for the wild boar and the domestic pig at municipal level (c).

Blood was collected directly from the heart into sterile tubes and sera obtained were frozen at -20ºC until being analyzed. Sera (n=1,272) were tested for ADV gE antibodies by means of an ELISA test (Chekit®PRV-gI, Bommelli, Switzerland) according to manufacturers´ recommendations. Sensitivity and specificity reach values of 95.2-98.9% and 97.8-99.5%, respectively (according to manufacturers´ data). ADV seroprevalence data were obtained from 54 of the 981 municipalities of CLM. We also

calculated ADV seroprevalence for each of the sampled hunting estates where 10 or more samples were available.

Wild boar management and habitat data

We visited 25 hunting estates in order to obtain field estimates of the relative abundance of wild boar, individuals´ aggregation and habitat variables. We chose a sample of sites that exhibited a range of management factors, variations in wild boar abundance and landscape diversity. These estates are located in the south-west of CLM (MT, GU and SM geographic regions, Figure 1). The habitat and management variables considered in the study were chosen on the basis of their likely epidemiological relevance and potential influence on the characteristics of wild boar and livestock (estate size, fencing, supplementary feeding, number of feeders and waterholes, presence of livestock and sanitary measures among others). We classified hunting estates as open (no fencing and almost no management) or fenced (fencing, supplementary feeding and sometimes translocations).

Wild boar relative abundance and aggregation estimates were based on dropping frequency counts (Vicente et al., 2004; Acevedo et al., in press). We calculated indexes of wild boar relative abundance per feeder and waterhole in each of the 25 hunting estates. Habitat use and structure in the study estates were recorded at points spaced every 200 m along linear transects (N=20 points per estate) and were used to calculate mean values for each estate. Habitat variables used for the epidemiological analyses are shown in Table 1.

Domestic pig data

ADV seroprevalence of the control and eradication campaign in CLM at farm level (1,652 out of 3,287 overall census) in 2004 were provided by the "Consejería de

Agricultura" of the regional government (JCCM) (n=264 municipalities). Also, data regarding the production system (open-air *vs*. indoor) and the number of pigs per farm were available. We calculated the density of domestic pig farms per hectare at municipal level. A mean ADV farm seroprevalence was calculated for each municipality. Serological tests were carried out by official laboratories, where a gE ELISA was performed (INGEZIM ADVGI, Ingenasa, Madrid, Spain).

Statistical analyses

Data were obtained from wild boars at estate and at individual level. The hunting estate is considered a discrete management unit where animals are under the same management and habitat conditions. Wild boar piglets were not included in the statistical analyses. Quantitative exploratory analyses of risk factors for ADV both in wild boar and domestic pig were obtained through a two-stage analysis.

In the case of the wild boar, statistical analyses were performed at an individual level for the wild boar (n=524). We firstly tested the association of each factor on ADV serostatus at individual level by means of Generalized Linear Mixed Models (GLIMMIX). The effect of sex and age was controlled in every model (Vicente et al., 2005, **Capítulo 1.1**). All the factors that captured the effect of any set of highly correlated variables for which $p<0.2$ were selected for inclusion in the final model (Table 1). We used a less restrictive procedure than $p<0.05$ due to the exploratory nature of the analyses. We performed GLIMMIX with the selected variables, retaining those variables with the lowest Akaike Information Criterion (AIC) (Burnham and Anderson, 1992). For this purposes, bivariate models were performed retaining the one with the lowest AIC and subsequently we used forward procedure (similarly to Quevedo et al., 2006). Hunting estate and geographic area were considered as random variables in order to control for local and regional influences.

In the case of the domestic pig, we firstly tested for the bivariate relationship between the continuous factors and ADV farm seroprevalences (%) by means of Spearman correlations (n=1,652 farms). In the case of categorical variables, a Kruskal-Wallis analysis was used. Variables that screened at least $p<0.2$ were selected for Generalized Linear Models (GLM) as previously explained. Confidence intervals for standard errors of seroprevalence were estimated with the expression S.E.95%C.I. = 1.96[p(1-p)]/n1/2 (Martin et al., 1987).

Spatial analyses

Spatial statistics were computed using GS+ software (Gamma Design Software, Plainwell, Michigan) for ADV seroprevalence data (both in wild boar and domestic pig) at municipality level. In a semivariogram, semivariance is plotted on the y-axis against lag distance (h) on the x-axis. The lag distance is the step-size used, and the active lag denotes the largest distance considered between points in the semivariance data set, though all data in the data set are included in the analysis. Using GS+ we calculated semivariance and then fitted curves to the semivariograms using spherical models as described by Isaacs and Srivastava (1989). The range, nugget, sill, and structural fraction for each analysis were determined from these models (see Rossi et al., 1992). In a spatial analysis of light distribution, the range (the distance along the x-axis at which the semivariogram function stops increasing) is indicative of the patch size of light gaps. The nugget, the y-intercept of the variogram, indicates the percentage of the overall variance not explained by space. The sill, or total sample variance, is the ordinate value at which the variogram becomes flat. The points analysed for the spatial association of ADV seroprevalence were the geographic centres of municipalities (i.e., municipality centroids). The use of centroids for these purposes has been previously reported (Getis, 1990; Hungerford, 1991; Austin and Weigel, 1992).

Table 1: Variables used for the risk factor analyses of ADV in wild boar grouped in different categories. Variables that were included in the final model ($p<0.2$) are marked (*). Statistic (F), degrees of freedom (DF) and the p value are shown.

Category	Variable	Num DF/Den DF	F	*p*
General	Estate type (categorical; open vs. fenced)	1/18.6	0.90	0.43
	Estate size (has)	1/21.3	1.41	0.25
	Years of fencing	1/20.9	1.40	0.25
	Fence (categorical; presence/absence)*	1/19.3	1.82	0.19
	Boundary fenced (%)*	1/33.9	13.06	0.001
	Boundary limiting with big hunting estates (%)	1/14	0.02	0.88
Wild boar data	Wild boar abundance index based on dropping counts	1/18.5	0.50	0.49
	Number of wild boar per feeder index	1/19	0.55	0.42
	Number of wild boar per waterhole index	1/15.7	0.01	0.94
	Wild boar aggregation index	1/20.8	0.68	0.42
Land uses	Evergreen oak (*Quercus ilex*) forest (%)	1/18.9	0.27	0.61
	Holm oak (*Quercus faginea*) forest (%)	1/24.1	0.27	0.61
	Pine (*Pinus* spp.) forest (%)	1/21.6	0.03	0.86
	Olive trees (%)	1/14.9	0.00	0.98
	Dehesa (savannah-like) habitat (%)	1/18.3	0.00	0.98
	Dehesa with pastures (%)	1/16.1	0.00	0.95
	Dehesa with scrubland (%)	1/17	0.06	0.80
	Pastures (%)	1/18.5	0.13	0.72
	Scrublands (%)	1/20.2	0.77	0.39
	Riverine habitat (%)	1/18	0.20	0.66
	Agricultural areas (%)	1/14.5	0.66	0.43
	Cultured hectares (%)	1/16.1	0.30	0.59
Habitat structure	Number of *Quercus* trees/10 m	1/18.9	0.78	0.39
	Number of *Quercus* spp. < 1m*	1/18.2	1.97	0.18
	Scrub diversity*	1/23.9	4.27	0.05
	Tree cover (%)*	1/17.5	2.58	0.13
	Scrubland > 50 cm cover (%)	1/17	0.15	0.71
	Scrubland < 50 cm cover (%)*	1/19.2	3.16	0.09
	Grass cover (%)	1/19	0.03	0.86
	Soil cover (%)*	1/18.1	2.48	0.13
	Soil compactness	1/15.4	0.00	0.97
Livestock Management	Domestic pig ADV seroprevalence (%)	1/15.5	0.80	0.38
	Number of feeders*	1/17.2	4.10	0.06
	Number of waterholes*	1/20.1	2.12	0.16
	Waterholes per hectare*	1/21.6	2.04	0.17
	Artificial feeding (categorical; presence/absence)	1/18.7	0.05	0.82

Results

Mean ADV seroprevalence for the analyzed wild boars was 36.63 ± 0.01 %. Wild boar ADV seroprevalences were medium-high in all the sampled areas but GJ, the northernmost area. ADV seroprevalences were higher than 50% in some municipalities located in SM, MT and TO areas (Figure 1). Mean ADV seroprevalence in the sampled

domestic pigs (n=58,814) was 21.1 ± 0.001%. Five hundred and ninety seven out of 1,652 analyzed farms (36.14%) were ADV seropositive. Mean ADV seroprevalences were higher in municipalities located in TO area than in the rest of the region, although some municipalities from other areas showed a high ADV seroprevalence (Figure 1).

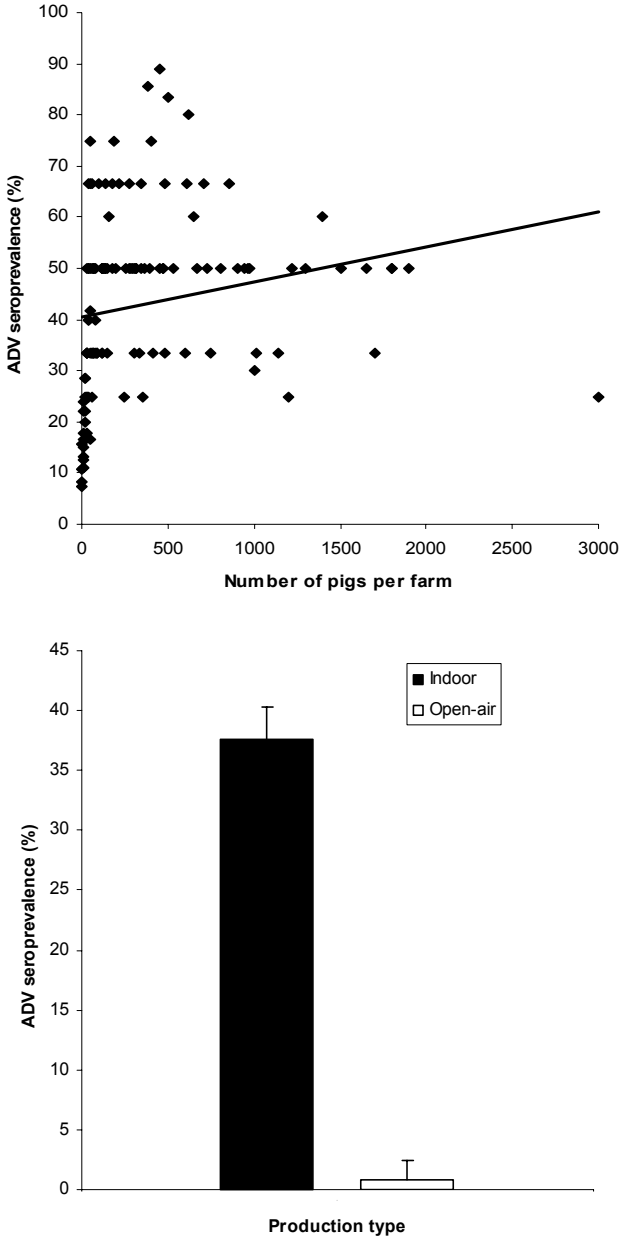


Figure 2: ADV seroprevalence in domestic pig farms through the number of pigs per farm and production type (± S.E.). Both variables were statistically influent on ADV seroprevalence.

Wild boar epidemiological analyses were performed for the 25 selected hunting estates while serologic data from all the analysed wild boars was used for the domestic pig epidemiological analysis. The most significant predictor of the probability of testing positive to ADV was age (Table 2). Adult wild boars presented a higher seroprevalence (71.9 ± 0.03 %) than sub-adults (46.8 ± 0.04 %) and juveniles (17.5 ± 0.04 %). Females presented statistically higher risk of testing positive (57.6 ± 0.03 %) than males (45.7 ± 0.03 %). Concerning land use and habitat structure, only the tree cover percentage showed a significant positive relationship with the individual risk of testing positive to ADV. The municipal seroprevalence of ADV in domestic pigs did not relate to the individual risk of testing positive to ADV in wild boars.

Concerning the domestic pig model (Table 2), higher ADV seroprevalences were observed along the increasing number of pigs per farm (Figure 2). Pig farm density at municipality level was correlated with the number of pigs per farm ($H=-0.078$, $p<0.01$). Indoor pig farms statistically showed higher seroprevalences (n=1529, 37.6 ± 0.03 %) than open-air farms (n=123, 0.81 ± 0.02 %).

Semivariogram fits based on ADV seroprevalence data both in wild boar and domestic pig evidenced spatial autocorrelation at municipality level. In the wild boar, spherical models provided excellent fits at lag distances of 6 km. At this scale, the range was 25.6 km, the sill was 0.94 and the nugget value was 0.001. In the domestic pig, spherical models provided best fits at lag distances of 3 km. At this scale, the range was less (9.5 km) than in the case of the wild boar model, the sill was 1.04 and the nugget value was 0.002.

Discussion

To our knowledge, this work is the first study dealing with the epidemiological interactions in ADV seroprevalences between the domestic pig and the wild boar in

such a large area. The study region represents a hot area of ADV seroprevalence in wild boar in Spanish mainland (Vicente et al., 2005, **Capítulo 1.1**). Subsequently, it also represents important knowledge on the relationships between the domestic pig and the wild boar ADV seroprevalences.

No relationship was found between wild boar and domestic pig ADV seroprevalences and the epidemiology of the virus in each other species. Here we noted that, while the highest wild boar densities are present in MT, SM and GU areas (in the south-west) (Höfle et al., 2004), the highest domestic pig farm densities in CLM are present in the centre of TO area (http://www.mapa.es/es/ganaderia/, Figure 1). The wild boar is scarcely present in the centre of TO area (Rosell and Herrero, 2002), a flat area mainly devoted to agriculture, and it is mainly located in fenced big hunting estates, overlapping with woodlands. This situation indicates that the spatial segregation between most domestic pig farms and wild boar populations makes it improbable any cross-transmission. Nevertheless, we measured ADV in terms of prevalence, and within each epidemiological context (wild and domestic, respectively), the factors that determine prevalence may differ, independently of any transmission event at the interface wild boar/domestic pig. For example, whereas habitat-related features revealed as influential in wild boar, factors related to density and farm size associated to increased prevalence in domestic pigs. This clearly indicates, as aforementioned, that new approaches are needed. ADV seroprevalences found in open-air domestic pig farms from CLM was low, although any statistical relationship would be difficulty detected attending to the sampling size (n=123). Extensive pig farming is much more important (in terms of number of farms and animals census) in other Spanish regions than in Castilla-La Mancha and a higher sampling effort should evaluate risk at the interface in theses areas (e. g in tuberculosis infection, Parra et al., 2005).

Table 2: Final models of ADV epidemiological risk analyses both in wild boar and domestic pig. Statistics (F value and Wald statistic, for GLIMMIX and GLMz, respectively), degrees of freedom (DF) and significance (p) are shown.

	Variable	Num DF/ Den DF	F	*p*
Wild boar model	Age	2/454	21.47	<0.001
	Sex	1/487	4.32	<0.05
	Tree cover (%)	1/20.2	4.60	<0.05

	Variable	DF	Wald	*p*
Domestic pig model	Number of pigs per farm	1	30.85	<0.001
	Production type	1	6.1	<0.05

The influence of age and sex in wild boar ADV epidemiology has been previously reported and discussed by the authors (Vicente et al., 2005, **Capítulo 1.1**). Apart from sex and age, the only factor found to statistically associate to the individual risk of testing positive in wild boar was the availability of woodlands. We can only speculate regarding this aspect. Similar results have been found for TB in wild boar across the study area (in particular, regarding the availability of *Quercus* spp. forest (Mediterranean hardwood, Vicente et al. in press, but see also Miller et al 2003). Woodland areas could provide shady, moist conditions under which ADV virus could survive for short periods in the environment. Alternatively, such habitats may become more important in an epidemiological sense if hosts positively select them and close contacts between them occur at these areas. *Quercus* spp. acorns from Mediterranean woodlands are intensively foraged by wild ungulates during autumn. If environmental contamination exists, wild boar and red deer feeding in the area (by rooting and muzzling while searching for acorns) could either ingest or inhale ADV viral particles. Acorn grazing is also a common practice in free-roaming Iberian pigs in large areas of Southern Spain and hence infectious interactions could occur.

Although not supported by this work, three main ways of ADV transmission between wild boars and domestic pigs can be suggested: i) via direct contact; ii) via consumption of infected carcasses; and iii) via aerosols. Contacts between both species could be increased in the case of open-air produced domestic pigs and in inappropriately restricted indoor farms, as suggested by Artois et al. (2002) in the case of classical swine fever (CSF). Infected pig carcasses available for domestic pigs and wild boars can suppose an increased risk of ADV and other pathogens transmission. Under adequate environmental conditions (low temperatures and high relative humidity), ADV can travel up to 80 km in aerosols (Christensen et al., 1990), for which airborne transmission must be considered as a possible way of transmission between both species. In the same way, transport of animals could imply stress that can lead to reactivation of latent ADV infections (Tanaka and Mannen, 2003). This fact could also suppose a risk of transmission via aerosol to domestic pigs or wild boars living near roads (Solymosi et al., 2004). Preventive measures of protection that avoid the contact between wild boars and pigs should be implemented in order to completely eradicate Aujeszky's disease from the domestic pig. Moreover, to reduce ADV seroprevalence in wild boars and the local risk of disease transmission to domestic pigs, control methods should be studied. This is especially true in the case of managed and densely populated hunting estates (Gortázar et al., 2006; Ruiz-Fons et al., 2006, **Capítulo 3**).

The spatial autocorrelation in the case of the domestic pig ADV seroprevalence had a low distance range. Thus, municipalities with similar ADV seroprevalence values are not more distant than 10 km. This fact could suggest that ADV spread and maintenance is dependent on the regional or municipal management systems, pig densities or distance among farms, which agrees with the epidemiological results in this work. Nevertheless, more precise information on farm geographical location based

analyses is needed in order to clarify this hypothesis. The spatial autocorrelation pattern for the wild boar showed a higher distance range in which ADV seroprevalence dissimilarities are low. Thus, ADV seroprevalence levels in municipalities separated less than 25 km are quite similar. Managed big hunting estates are concentrated in mountainous areas of the south-west of the study region. Open non-managed big hunting estates, usually of public owning, and agricultural areas are placed between fenced estates. This particular situation could imply that ADV circulates among closely located estates and large open hunting estates or agriculture areas could serve as partial barriers for ADV transmission. Moreover, as ADV seems to be related to the current management systems (Vicente et al., 2005, **Capítulo 1.1**) and to wild boar abundance (Acevedo et al., in press), its distribution could be limited outside the highly populated areas, as suggested for the classical swine fever (CSF) in wild boars from France (Rossi et al., 2005). Nevertheless, in order to understand ADV spatial distribution in wild boar populations from the study area, a random sampling would be necessary.

The main finding of this research is that, at least at the study scale, no evidence of interaction in the epidemiology of AD between the domestic pig and the wild boar was found. These results reinforce the idea that more studies are needed, especially molecular approaches and probably, local scale approaches to the epidemiology of ADV in areas where the risk of transmission between wild boar and domestic pig is high, such as open-air Iberian pig systems.

Conclusion

At the study scale, no signs of any associations between wild boar and domestic pig ADV seroprevalence were evidenced in this study. As seroprevalence could not be indicative of wild boar/domestic pig interaction at the interface, more research should

focus in the use molecular and finer scales, taking special attention to open-air Iberian pig systems to definitively elucidate the epidemiological role of both groups of suids.

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Capítulo 5

Respuesta de anticuerpos en rayones (*Sus scrofa*) vacunados contra el virus de la enfermedad de Aujeszky

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Antibody response of wild boar (Sus scrofa) piglets vaccinated against Aujeszky´s disease virus

Enviado a: ***Research in Veterinary Science***

Resumen

La respuesta humoral del jabalí europeo al virus de la enfermedad de Aujeszky (ADV) fue asesorada a través de un ensayo vacunal. Trece rayones fueron vacunados y revacunados con una vacuna deletérea en la glicoproteína E. Los sueros fueron analizados para la presencia de anticuerpos contra gE y gB del ADV por medio de dos tests ELISA. Antes de la vacunación, cuatro y ocho de los animales fueron positivos a anticuerpos contra gE y gB, respectivamente. Los títulos de anticuerpos contra el ADV se determinaron mediante un ensayo de neutralización del virus. El título medio de anticuerpos aumentó después de la vacunación y experimentó una mejora tras la revacunación. No se observaron diferencias significativas en la respuesta humoral entre animales vacunados en presencia o ausencia de anticuerpos anti-gB de origen materno. Nuestros resultados muestran una respuesta humoral similar o mayor que la descrita para lechones.

Abstract

The humoral response of European wild boar to Aujeszky´s disease virus (ADV) was assessed by means of a vaccination trial. Thirteen wild boar piglets were vaccinated and revaccinated with a glycoprotein E deleted vaccine. Sera were tested for the presence of antibodies against ADV gE and gB by means of two ELISA tests. Before vaccination four and eight of the animals were positive for gE and gB antibodies, respectively. ADV antibody titters were determined by means of a viral neutralisation assay. Mean antibody titter increased after vaccination and experimented a booster after revaccination. No significant differences were seen in humoral response between animals vaccinated in presence or absence of maternally-derived anti-gB antibodies. Our results showed a humoral response similar or higher to that reported for domestic piglets.

Keywords: Control; Immune response; Maternal antibodies; Vaccination; Wildlife.

Introduction

Aujeszky´s disease (AD) is one of the major diseases of pigs and is subjected to eradication campaigns in many countries (Moynagh, 1997). The aetiological agent is AD virus (ADV), which natural hosts are wild and domestic swine, the only known species that can survive an infection with a wild-type strain (Kluge et al., 1999). Under some circumstances, for example extensive pig production, the chance of a contact between wild boars and domestic pigs is increased. For instance, ADV transmission from wild boars to domestic pigs has been reported to be the origin of an AD outbreak in the French Department of Loiret (Hars and Rossi, 2005). ADV seroprevalence in Spanish wild boars has been estimated to be around 40% (Vicente et al., 2005, **Capítulo 1.1**) and wild boar densities have increased during the last three decades in Spain (Saez-Royuela and Tellería, 1986). Also, game management systems in Central and Southern Spain nowadays often include fencing, watering and feeding but sanitary measures have not been implemented to match this management. In addition, wild boar translocations are frequent in order to increase the number of animals for hunting purposes.

In domestic pigs, control of AD is achieved through vaccination with glycoprotein E (gE) deleted ADV vaccines. Therefore, vaccination could be a system to control ADV infection in farmed and translocated wild boars, as eradication campaigns in the domestic pig are based on it (Müller et al., 2003). As a matter of fact, some Spanish estate and farm managers use commercial attenuated vaccines against ADV in wild boars to reduce the chance of transporting the infection because of translocated animals. In addition, mortality associated to ADV infection in wild boars has been previously reported (Gortázar et al., 2002). However, the immune response of wild boars to ADV

vaccination is unknown. The aim of the present study was to evaluate the humoral response of wild boars after ADV vaccination.

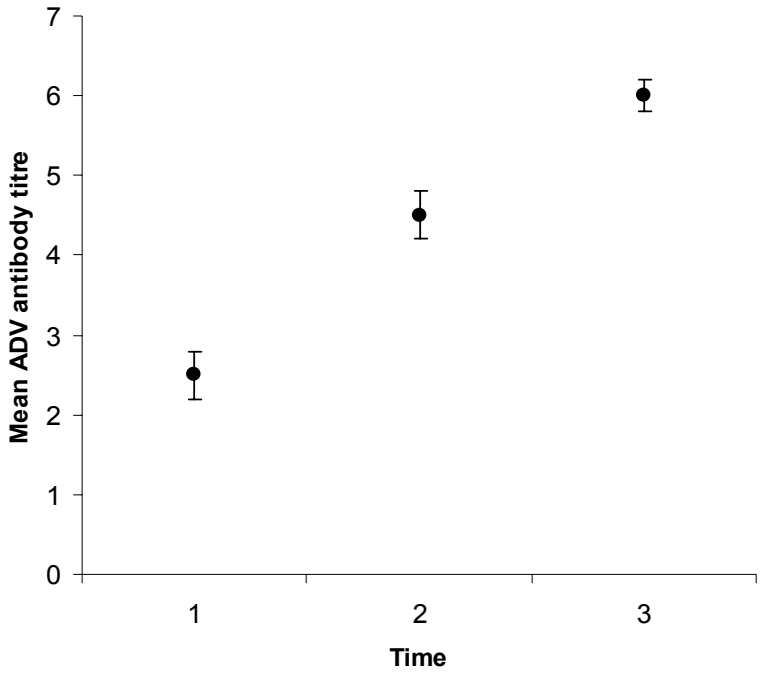
Material and methods

During the summer 2004, 13 free-living 3 to 5 months-old wild boars (age estimated by tooth eruption pattern (Saenz de Buruaga et al., 1991)) were captured in a hunting estate located in the province of "Ciudad Real" (south-central Spain). Captures were carried out by gamekeepers with portable capture boxes. Animals were placed together in conditioned areas, identified with an electronic microchip inserted subcutaneously below the right ear and allowed to acclimatise for one week. Also, 24 adult captured wild boars were placed in an adjoining enclosure only separated from that of the piglets by a single fence. Piglets were intramuscularly vaccinated with an attenuated ADV gE deleted (gE^-) strain (Porcilis Begonia, Intervet Laboratories, The Netherlands), using a dose of $10^{5,5}$ $TCID_{50}$ in 2 ml. Piglets were revaccinated with the same vaccine and dose 2 months later. Piglets were bled by cervical puncture before the first vaccination, just before revaccination, and three months after revaccination. Adult wild boars were also bled in the same day of piglet vaccination. Piglet sera were analysed for antibodies against ADV gE and gB by means of ELISA tests (Chekit®PRV-gI and Chekit®PRV-gB, Bommeli, Switzerland) according to the manufacturer's instructions. Adult wild boar sera were only tested for gE antibodies. Piglet sera were also tested by the viral neutralisation assay according to the OIE recommendations (Anonymous, 2004). Statistical analysis of the mean antibody titre results was done by ANOVA.

Results

When captured, 4 wild boar piglets were seropositive to gE and 8 had anti-gB antibodies. After the first vaccination all piglets became gB seropositive and remained so until the end of the study. At the end of the study all piglets were gE negative indicating the maternal origin of those antibodies. ADV gE antibodies were detected in 13 of the adult wild boars (n=24). Just before the first vaccination, gB seropositive captured wild boar piglets had a mean virus neutralisation titre of 2.5 (log_2 titre). No statistical differences were seen regarding the humoral response between animals vaccinated in presence or absence of anti-gB antibodies. Thus, mean virus neutralisation titre after first vaccination was 4.5 and 6 after revaccination (Fig. 1). This increase was statistically significant (F=17.61, P<0.001).

Figure 1. Mean virus neutralisation antibody titres (log_2 titre) before vaccination (time 1), after vaccination (time 2) and after revaccination (time 3). The increase in antibody titre was statistically significant (F=17.61, P<0.001).



Discussion

There are evidences that wild boars may transmit ADV to domestic pigs (Hars and Rossi, 2005). Therefore, it is advisable to figure out strategies to control ADV in wild boars, particularly when this species is raised in semi-captivity and translocated between hunting estates. Vaccination could be one of these control methods as occurs with the domestic pig. The present experiment is to our knowledge the first report on the humoral response of wild boars to ADV vaccination.

The humoral response to vaccination did not seem to be impaired by the presence of low levels of maternal-derived antibodies as previously reported for twice vaccinated domestic piglets with similar maternal-derived antibody titres (Bouma et al., 1997). Although the number of examined boars was small and such effect can not be ruled out if examined on a population scale.

ADV circulates among the wild boars in the study hunting estate. Similar virus neutralisation titters did not completely protect domestic piglets against Aujeszky´s disease. Nevertheless, virus excretion duration and titres were lower in gE^- vaccinated piglets than in control ones (Vannier at al., 1995). Our results showed that vaccinated wild boars developed a humoral response similar or higher to that of conventional pigs (Vannier et al., 1995) and neutralising antibodies to the virus. Also, revaccination induced a booster in neutralising antibodies.

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Capítulo 6

Síntesis y conclusiones

SÍNTESIS

Este capítulo sintetiza los resultados más relevantes de los capítulos en los que se ha distribuido la presente Tesis, con la excepción de la revisión de enfermedades víricas del jabalí presentada en el Capítulo 1.

Dado el creciente interés de las especies cinegéticas, y el consiguiente incremento de los beneficios económicos que se pueden obtener de ellas, la caza tradicional en la que se aprovechaba lo que daba el monte está poco a poco siendo transformada en sistemas productivos. Estos nuevos sistemas de producción cinegética suelen conllevar un incremento en la densidad de animales mediante el vallado cinegético, la alimentación artificial y los traslados tanto legales como ilegales. Se crean, de este modo, situaciones semejantes a los sistemas de producción de especies de abasto en los que la gran diferencia es, sin lugar a dudas, la escasa presencia de las medidas de control sanitario. Entre las especies de caza producidas en nuestro país, y de gran importancia en el resto de Europa, se encuentra el jabalí. Así, el presente trabajo de Tesis pretende evaluar el riesgo sanitario que representan los sistemas de manejo cinegético para el jabalí, tomando como modelo la enfermedad de Aujeszky.

Los jabalíes del centro sur de la Península Ibérica presentan elevadas prevalencias de infección por el virus de la enfermedad de Aujeszky

Estudios previos señalaban la presencia del virus de la enfermedad de Aujeszky (VEA) en el jabalí en España y la presencia de casos clínicos asociados a esta enfermedad. Para ampliar este conocimiento se muestrearon jabalíes procedentes de diferentes zonas de la España peninsular: Principado de Asturias, Aragón, Castilla-La Mancha, Extremadura, Andalucía y Castilla y León (Burgos y Ávila). La detección de anticuerpos contra el VEA en las poblaciones peninsulares de jabalíes se determinó mediante el uso de una técnica serológica (ELISA). Esta técnica fue desarrollada

inicialmente para el cerdo doméstico, aunque ha sido ampliamente usada en el jabalí. Los resultados arrojaron la presencia de anticuerpos en los jabalíes del centro sur de la Península, y en un animal procedente del sur de Aragón. Los niveles de seroprevalencia fueron muy elevados en muchas de las fincas muestreadas, presentando valores significativamente más elevados en las fincas sometidas a un mayor grado de manejo que en aquellas fincas escasamente manejadas. Esto se asoció principalmente a la abundancia de jabalíes, que resultó significativamente mayor en las fincas manejadas que en aquellas no manejadas. Los sistemas de manejo facilitan el incremento de la abundancia de jabalíes a través de la alimentación artificial y la introducción de animales procedentes de otras fincas. Así mismo, la alimentación artificial y la disponibilidad de agua en pocos puntos pueden conducir a un incremento de la agregación de animales, con el consecuente incremento de la tasa de contacto entre animales infectados y animales susceptibles.

Se observó un patrón creciente de seroprevalencia con la edad, asociado seguramente a la mayor probabilidad de contacto con el VEA. Las hembras presentaron valores mayores de seroprevalencia que los machos, lo que pudo ser debido tanto al diferente comportamiento social de las hembras, más gregarias que los machos, como a las diferencias en la maduración sexual con los machos. En este sentido, la seroprevalencia no difirió significativamente a lo largo del año en las hembras, mientras que en los machos se observó un pico máximo de seroprevalencia tras la época de celo. Los valores bajos de seroprevalencia en los machos pueden ser debidos a la incorporación de machos jóvenes a la clase de edad de más de un año, a partir de la cual fueron considerados los animales para los análisis estadísticos. Se especuló que la transmisión entre las hembras, de mayor comportamiento gregario, podría ser principalmente por rutas directas, mientras que la transmisión entre hembras y machos

podría ser fundamentalmente venérea, como se ha sugerido para los jabalíes híbridos en Norteamérica.

Para confirmar el grado de infección por el VEA obtenido a través de los resultados serológicos, se tomaron muestras de 192 jabalíes procedentes de la zona previamente identificada de presencia del VEA. En estos animales se analizó la presencia de genoma del virus en tonsilas y ganglios nerviosos trigéminos a través de una técnica molecular (PCR anidada) específica para la glicoproteína de membrana B del VEA. El 30 % de los animales presentaron presencia del virus en alguno de los tejidos analizados o en ambos a la vez. La vía de transmisión oral/nasal en los jabalíes se puso de manifiesto en los jabalíes del centro sur peninsular al encontrar la presencia del virus en un porcentaje elevado de las tonsilas analizadas. Con la finalidad de determinar el estado de circulación del VEA, se clasificó el curso de la infección en base a la ausencia del virus o a su presencia en tonsilas, en trigéminos o en ambos a la vez. Los resultados reflejaron la sensibilidad de los animales adultos a una primoinfección por el virus al detectarse un gran número de esta clase de edad recientemente infectados por el virus (presencia del virus sólo en tonsilas). El porcentaje de animales positivos en trigémino o en tonsila y trigémino a la vez fue mucho menor. Los resultados de la clasificación del estado de infección por el virus se compararon con los resultados obtenidos del análisis serológico, poniendo en evidencia que los animales con presencia del virus en trigémino y en tonsila y trigémino a la vez presentaban niveles de anticuerpos circulantes detectables por serología. Sin embargo, un gran porcentaje de los animales que presentaron el virus solamente en las tonsilas no presentaron niveles de anticuerpos detectables en la técnica serológica usada. Igualmente, un elevado porcentaje de los animales negativos a la presencia del virus resultaron positivos a la presencia de anticuerpos contra el virus. Estos resultados

pueden explicarse en base a la naturaleza atenuada demostrada de las cepas del VEA circulantes en las poblaciones de jabalíes. La restricción de cepas atenuadas del VEA a las zonas de entrada podría indicar que en el caso de transmisión venérea el virus puede quedar acantonado en ganglios nerviosos de la zona lumbar, como fue demostrado para jabalíes híbridos de Norteamérica. En el trabajo no se analizaron los ganglios nerviosos sacros, por lo que esta hipótesis deberá ser posteriormente confirmada. Así, los animales seropositivos pero sin presencia del virus en tonsilas y trigéminos podrían haber contactado con el virus por vía venérea o haber eliminado el virus eficazmente.

El diagnóstico individual de la enfermedad de Aujeszky tanto en cerdos domésticos como en jabalíes suele realizarse mediante el uso de técnicas serológicas. Sin embargo, los resultados obtenidos muestran un 45 % de los animales con presencia del virus y sin reacción serológica evidente. Este resultado puede ser de gran importancia de cara al transporte de jabalíes de zonas con presencia del virus a zonas en las que no se ha detectado el virus cuando se utilizan técnicas serológicas para el diagnóstico.

La tasa de reproducción de las hembras de jabalí es menor bajo condiciones de manejo que en poblaciones naturales

El efecto de los sistemas de producción cinegética sobre la tasa de reproducción de las hembras de jabalí se evaluó mediante la recolección de úteros y ovarios en fincas manejadas y no manejadas. Se calcularon la tasa de ovulación, el tamaño de camada y un índice de absorción parcial como el número de cuerpos lúteos en ambos ovarios menos el número de fetos en útero. Los resultados evidenciaron medias de la tasa de ovulación y del tamaño de camada más bajas en aquellas fincas con grados de manejo elevado que en las fincas en las que los jabalíes no estaban sometidos a manejo alguno. Lo contrario fue observado para el índice de reabsorción parcial. Las tasas reproductivas

de las hembras de jabalí de las fincas manejadas solamente fueron comparables con las obtenidas para jabalíes del Parque Nacional de Doñana en época de sequía. Así mismo, se averiguó la asociación estadística entre los parámetros reproductivos de las hembras de jabalí y diferentes patógenos de conocido efecto reproductivo obtenidos mediante análisis serológico. Los resultados no mostraron relación alguna entre los diferentes agentes infecciosos (incluyendo el virus de la enfermedad de Aujeszky) y los parámetros reproductivos a excepción del parvovirus porcino y *Toxoplasma gondii*.

Los resultados de seroprevalencia obtenidos para los diferentes patógenos permitieron calcular un índice que fuese indicativo de la tasa de contacto con los diferentes patógenos analizados a nivel individual. Así, se observó que el número de agentes infecciosos con los que los animales habían tenido contacto fue mayor en las fincas manejadas que en aquellas no manejadas. Así, nuevamente se demuestra que los sistemas de producción cinegética incrementan el riesgo de contacto de los animales infectados y los animales susceptibles.

El jabalí no representa un riesgo de transmisión del virus de la enfermedad de Aujeszky para el cerdo doméstico en Castilla-La Mancha

El jabalí es capaz de mantener la infección por el VEA por sí solo, sin la intervención del porcino doméstico. Así, la finalidad de este estudio fue la de determinar el riesgo epidemiológico de la presencia de infección por el VEA en los jabalíes presentes en los términos municipales con presencia de porcino doméstico. Para ello, se estudió el efecto de diferentes factores de riesgo sobre las explotaciones de cerdo y se incluyeron los niveles de seroprevalencia en los jabalíes muestreados en el término municipal de localización de las explotaciones. El estudio se realizó para un gran número de las explotaciones porcinas de Castilla-La Mancha.

Los resultados observados señalaron la presencia del VEA en un elevado porcentaje de las granjas de cerdo doméstico de la región. Factores como el número de cerdos por explotación o el régimen productivo resultaron influyentes sobre el riesgo de las granjas de porcino a ser positivas al VEA. Se observó un incremento de la seroprevalencia del virus paralelo al incremento en el número de cerdos presentes en la explotación. Las diferencias de seroprevalencia del VEA entre las granjas de producción intensiva y las de producción extensiva o semi-extensiva fueron significativas. Así, se observó un nivel muy bajo de seroprevalencia en las explotaciones extensivas, mientras los niveles fueron muy elevados en las intensivas.

Cuando la seroprevalencia del VEA en los jabalíes del término municipal de localización de las explotaciones fue analizado en relación con el riesgo de las explotaciones a tener presencia del virus, no se observó ninguna influencia significativa. Se concluyó que, al menos a esta escala de estudio, el jabalí no supone un riesgo para el mantenimiento y la circulación del virus en las explotaciones porcinas. Esto también puede ser debido a que la mayor concentración de explotaciones porcinas se localiza en el centro de la provincia de Toledo, mientras que el jabalí se distribuye principalmente en zonas más montañosas de la zona de estudio. Sin embargo, las explotaciones de porcino en extensivo suelen localizarse en aquellas zonas en las que la presencia de jabalíes es elevada. Al mismo tiempo, los sistemas de producción en extensivo no ofrecen ningún tipo de protección efectiva que evite el contacto entre cerdos y jabalíes. En estos casos la transmisión de enfermedades entre ambos suidos puede verse incrementada. En estos casos el jabalí podría jugar un papel negativo con respecto a la eficacia de las campañas de erradicación de la enfermedad de Aujeszky en el porcino.

Los niveles de seroprevalencia observados a nivel de términos municipales de Castilla-La Mancha fueron analizados con la finalidad de determinar la asociación

espacial entre niveles de seroprevalencia similares. Los resultados del análisis espacial tanto en el porcino doméstico como en el jabalí señalaron distancias con niveles similares no superiores a 10 km y a 25 km, respectivamente. En el caso del porcino, este resultado puede estar en concordancia con lo observado en el análisis de los factores de riesgo. Así, se podría concluir que los factores de riesgo para la circulación y el mantenimiento del virus en las explotaciones porcinas son más dependientes de los sistemas de manejo de los animales que de la posibilidad de contacto con jabalíes infectados. En el caso del jabalí, las mayores distancias observadas de asociación de las seroprevalencias pueden poner de manifiesto la circulación del virus en áreas concretas, y el papel de grandes fincas abiertas o grandes áreas agrícolas como barreras parciales para la dispersión de los jabalíes y, por lo tanto, de la transmisión del VEA entre unas y otras zonas.

La vacunación puede ser un método de control de la enfermedad de Aujeszky en los jabalíes

En la actualidad no existe información disponible sobre métodos de control de la enfermedad de Aujeszky en el jabalí. Con la finalidad de determinar la eficacia de la vacunación contra el VEA en los jabalíes, se desarrolló un experimento de vacunación en rayones. Se vacunaron y revacunaron 13 rayones con una vacuna comercial basada en una cepa atenuada del VEA en la que la glicoproteína E está ausente, lo que permite diferenciar serológicamente entre animales vacunados y animales infectados por cepas de campo. La respuesta inmune en los animales vacunados se cuantificó mediante la técnica de seroneutralización del virus. Los niveles de anticuerpos neutralizantes detectados alcanzaron valores similares o superiores a los obtenidos tras la vacunación de lechones. A pesar de que no se hizo ninguna prueba de desafío, se concluyó que los niveles de anticuerpos obtenidos podrían representar una protección frente al virus de

campo similar a la observada en el porcino doméstico. Además, se observó que aplicando un protocolo de revacunación, la respuesta humoral incrementaba la producción de anticuerpos hasta niveles muy elevados. Esta respuesta humoral no se vio sin embargo afectada por la presencia de niveles bajos de anticuerpos maternales.

Se concluyó que la vacunación con cepas comerciales atenuadas del VEA podría ser un sistema eficaz de control de la enfermedad en aquellas poblaciones de jabalíes en semi-cautividad y en producción en granjas.

CONCLUSIONES

1. El virus de la enfermedad de Aujeszky está ampliamente difundido en las poblaciones de jabalíes del centro y sur de la Península Ibérica, y los niveles de infección y contacto son elevados. En cambio, apenas se ha detectado su presencia en las poblaciones del norte peninsular.

2. La producción cinegética conlleva elevadas prevalencias del virus de la enfermedad de Aujeszky en los jabalíes. Este hecho puede tener consecuencias no sólo para el estado sanitario de los jabalíes, sino también de cara a otras especies domésticas y silvestres que pueden infectarse con el virus. De especial mención es el riesgo para el lince Ibérico, que cohabita con jabalíes en los que la seroprevalencia del virus es muy elevada, y que podría contagiarse al consumir jabalíes infectados.

3. Las hembras y los animales adultos presentan tasas más elevadas de contacto con el virus de la enfermedad de Aujeszky que los machos y los animales más jóvenes.

4. El diagnóstico serológico para determinar el estado individual de infección o contacto con el virus de la enfermedad de Aujeszky parece no ser suficiente dada la presencia de animales infectados por el virus y sin niveles detectables de anticuerpos. Este resultado es de especial relevancia en traslados de jabalíes.

5. La tasa reproductiva hallada en las hembras de jabalí de fincas muy manejadas es de las más bajas de Europa y sólo comparable a las situaciones extremas de sequía observadas en el sur peninsular. En este contexto, algunos agentes infecciosos pueden modelar la dinámica poblacional de los jabalíes a través

de su efecto sobre la función reproductora. Los jabalíes de fincas intensamente manejadas presentan mayor contacto con enfermedades que los de fincas abiertas.

6. La presencia de elevadas tasas de seroprevalencia en las poblaciones de jabalíes del centro sur peninsular no representa un riesgo para el porcino doméstico, al menos a la escala del estudio. Sin embargo, el riesgo local de transmisión del virus de la enfermedad de Aujeszky al porcino doméstico en extensivo debe tenerse en cuenta de cara a la erradicación de la enfermedad.

7. Los métodos de control de la enfermedad de Aujeszky parecen necesarios bajo el panorama de evolución de la producción cinegética del jabalí, particularmente en granjas o ante traslados. En este contexto, la vacunación con cepas atenuadas del virus de la enfermedad de Aujeszky proporciona una buena respuesta inmune humoral en jabalíes en semi-cautividad.